P14-01
Citocline attenuates phospholipase A2 stimulation and hydroxyl radical generation in transient cerebral ischemia
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Citocline (CDP-choline) has undergone 13 phase III clinical stroke trials, and is being evaluated for treatment of Alzheimer’s and Parkinson’s diseases. Phospholipid degradation and generation of reactive oxygen species (ROS) are major factors causing neuronal injury in CNS trauma and neurodegenerative diseases. Oxidative metabolism of arachidonic acid, which is released by the action of phospholipases, contributes to ROS generation. Here, we examined the effect of citocline on phospholipase A2 (PLA2) activity in relationship to attenuating the hydroxyl radical (OH·) generation after transient forebrain ischemia of gerbil. A 10-min transient forebrain ischemia was induced by bilateral carotid artery occlusion in male Mongolian gerbils with reperfusion up to 24 h. Citocline (500 mg/kg IP in saline) was given to gerbils at 0- and 3-hr reperfusion. PLA2 activity in hippocampal membrane and mitochondrial fractions was determined. OH· were determined by salicylate trapping and HPLC with electrochemical detection. The predominant form of PLA2 in gerbil hippocampus required millimolar Ca2+ concentrations, characteristic of 14-kDa secretory PLA2. PLA2 activity increased in both membrane and mitochondrial fractions after transient cerebral ischemia in gerbil, which was significantly attenuated by citocline treatment. In vitro, citocline and its components cytidine and choline had no effect on PLA2 activity, and thus citocline is not as such a PLA2 inhibitor. Ischemia/reperfusion resulted in significant OH· generation and citocline significantly attenuated their formation. These results suggest that citocline inhibits the stimulation of PLA2 and attenuates ROS formation after transient cerebral ischemia.

Keywords: CDP-choline, lipid peroxidation, neuroprotection, phospholipids, reactive oxygen species.

P14-02
NMDA receptors expression and immunoreactivity in experimental cerebral ischemia and hemorrhage
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NMDA receptors are a key biomarker of neurotoxicity underlying the stroke that leads to neuronal receptor damage in cerebral ischemia but not hemorrhage (1). We compared NMDA receptors expression and immunoreactivity in experimental cerebral ischemia and hemorrhage. Adult male Sprague-Dawley rats (n = 18) were subjected to 60 min cerebral artery occlusion (MCAo). Intracerebral hemorrhage was induced by injection of lysed blood cells (n = 12). Animals were sacrificed at 0, 24, and 72 h after surgeries, expression of NR2A/2B mRNA by RT-PCT were assessed. NR2A/2B subunits immunoreactivity in cortex and hippocampus and their autoantibodies appearance in the blood of animals were compared. In infarced cortex of MCAo rats the dynamic of NR2A/2B expression was observed: it was reduced at 0 h, up-regulated at 24 h, and followed decrease to the 72 h after reperfusion. At the same time expressions in both structures of rats with induced hemorrhage were reduced. Compared with rats with hemorrhage, NR2A/2B immunoreactivity decreased significantly (p < 0.05) at 24 h after induced cerebral ischemia. There are no changes of NR2A/2B cortex/hippocampus immunoreactivity and their autoantibodies concentrations detected in rats with induced hemorrhage. While significantly elevated levels of NR2A/2B autoantibodies in the blood of MCAo rats after 72 h of post-ischemic reperfusion were measured. Our results indicate the up-regulated NR2A/2B subunit expression and their autoantibodies appearance independently associated with cerebral ischemia.

Keywords: glutamate receptor, hemorrhage, immunooasays, ischemia.

Reference

P14-03
Reactive species and apoptotic cell death in spinal cord injury
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We previously demonstrated that apoptotic cell death follows traumatic spinal cord injury (SCI) and that the concentrations of several reactive species (RS) significantly increase after SCI. This study is to test whether SCI-elevated RS contribute to apoptosis and to explore the pathways for RS-inducing apoptosis by administering RS into the rat spinal cord at the concentration and duration produced by SCI. Hydrogen peroxide was administered into the cord through a microcatheter, S-morpholinosydnonimine (SIN-1), a donor of superoxide and nitric oxide) was similarly administered to produce peroxynitrite, and Fenton’s reagents were administered separately through two microdialysis fibers to generate hydroxyl radicals in the cord. Fragmented DNA was visualized by TUNEL staining and double staining with TUNEL and neuron-specific enolase (NSE) antibodies to identify TUNEL-positive neurons. TUNEL-positive cells or neurons were counted to compare the levels of DNA fragmentation among RS-injured, ACsf control and Mn (III) tetrakis (benzoic acid) porphyrin (MnTBP) – a cell-permeable superoxide dismutase mimetic and a broad spectrum reactive species scavenger-treated groups. Our results show that all three species at the level produced by SCI induced significantly more cell death by both necrosis and apoptosis than did controls, demonstrating that these reactive species are important secondary damaging agents. We also observed significantly more active caspase-3-positive cells in the RS-injured sections than controls by counting active caspase-3 immunostained cells, suggesting that caspase-3 activation is involved in these RS-induced apoptosis. MnTBP significantly reduced RS-induced apoptosis, total cell death and caspase-3 activation.

Keywords: apoptosis, caspases, free radicals, microdialysis, spinal cord trauma.

P14-04
A gene cloning for goldfish optic nerve regrowth and refinement
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We have previously reported fast and slow recovery phases of goldfish behavior after optic nerve transection, which are thought to correlate with the arrival of optic axons at the tectum 3–4 weeks after axotomy and synaptic refinement of axon terminals in the tectum 3–4 months after axotomy, respectively. In the present study, we identified genes whose expression is specifically upregulated in the retina in the early stage and in the tectum in the late stage of optic nerve regeneration. The cDNA libraries constructed from goldfish retina or tectum were screened by differential hybridization with cDNA probes derived from retina and tectum of axotomized or normal goldfish. Of six cDNA clones isolated from the retinal library, one clone was identified as Na,K-ATPase catalytic subunit alpha-3 isoform. The expression level of the mRNA was increased at 2 days and peaked at 5–10 days after axotomy. Both in situ hybridization and immunohistochemical staining have revealed that the retinal location of this transient increase of Na,K-ATPase alpha-3 subunit is only in the ganglion cell layer and nerve fiber layer. In an explant culture system, a low concentration of ouabain (50–100 nM) completely blocked the spontaneous neurite outgrowth from the lesioned retina. These data indicate that upregulation of the Na,K-ATPase alpha-3 subunit is involved in the regrowth of ganglion cell axons. Ten positive cDNA clones were isolated from the tectal library. Their expression patterns were detected in the tectum during refinement of goldfish retinotectal topography after optic nerve lesion. Thus, the goldfish visual system is very useful for studying the molecular mechanism of CNS regeneration.

Keywords: axon regeneration, neurite outgrowth, regeneration, retina, visual pathway.
P14-05
Uppregulation of transglutaminase and its possible role in goldfish optic nerve regeneration
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Unlike the mammalian CNS, lower vertebrates including fish and amphibians can successfully regenerate axons throughout their life. Therefore, the goldfish optic nerve can regenerate after transection. Transglutaminase (TG) is one enzyme that is upregulated after nerve injury. To investigate the molecular mechanism of fish TG during optic nerve regeneration, we isolated a cDNA clone of TG from a cDNA library, which was constructed from goldfish retina 5 days after optic nerve transection. Accordingly, the predicted TG protein comprised 678 amino acids. We made a recombinant protein and thereby obtained an antiserum against TG. We further examined the enzyme activity, protein, and mRNA expression in the retina after axotomy. TG enzyme activity and mRNA content increased after optic nerve transection, peaking at 20 days. In situ hybridization and immunohistochemical studies showed that the increase in TG expression was localized only in ganglion cells of the regenerating retina. Using an in vitro culture system, neurite outgrowth from matured retinal explant was clearly inhibited by adding anti-TG serum or TG inhibitors. Using an in vivo assay system, the retinotectal connection traced with WGA-HRP was clearly blocked by daily treatment of TG inhibitors into the goldfish orbit for 30 days. These results indicate that upregulation of TG is indispensable for regrowth of optic nerve fibres.

Keywords: axon regeneration, neurite outgrowth, regeneration, retina, visual pathway.

P14-06
A different expression of galectin-3 in the fish and the rat retinas after optic nerve injury
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The retinal ganglion cells (RGCs) of goldfish can survive and regrow their axons after optic nerve injury, whereas RGCs of rat cannot survive and become apoptotic 7 days after optic nerve injury. Galectin, a β-galactoside binding lectin, which is classified at least 14 subfamilies, has been implicated for various biological phenomena. Recently, among of them galectin-3 was identified as a candidate substance for promoting neurite outgrowth. Therefore, in the present study we compared the galectin-3 expression in the goldfish and rat retinas after optic nerve crush. An immunohistochemical study revealed that the fish and rat RGCs were specifically labeled with galectin-3 antibody after injury. However, the time course of galectin-3 labeling after optic nerve crush was different from each other. In goldfish retina, the increase of expression of galectin-3 was initiated at 3 days, peaked at 7–20 days and continued for 50 days after injury. In rat retina, the increase of expression was initiated at 2–3 days, peaked at 5–6 days and suddenly lost at 7–9 days after optic nerve injury. The immunohistochemical data were quantitatively confirmed by Western blotting analysis. The long-lasting increase of galectin-3 expression in the goldfish RGCs reflects a regenerative potential of fish to CNS injury. The transient increase of galectin-3 expression in the rat RGCs is not thoroughly understood at present. Galectin-3 will offer some clues for solving the difference in CNS regeneration between fish and mammals.

Keywords: axon regeneration, neurite outgrowth, regeneration, retina, visual pathway.

P14-07
Antia apoptotic profile during goldfish optic nerve regeneration
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Mature neurons in the CNS of mammals fail to regrow their axons after injury. Unlike mammals, the CNS neurons of lower vertebrates including fish and amphibians retain the capacity to generate axons throughout their life. Thus, retinal ganglion cells (RGCs) in the goldfish can successfully regrow their axons to re-innervate the tectum, in contrast to mammals where RGCs become apoptotic following injury. Focusing on this difference between fish and mammals after optic nerve injury, we compared both goldfish and rat RGCs after optic nerve crush in respect to cell death and survival signals. Fish RGCs could survive indefinitely, whereas rat RGCs were gradually lost after 6 days of treatment. The enzyme activity of caspase-3, a major executioner of apoptotic signals, in rat retina was specifically activated, accompanying the cell loss. By contrast, the caspase-3 activity in the fish retina was reduced during 10–20 days of treatment. We further measured levels of antiapoptotic Bel-2 and proapoptotic Bax proteins in both goldfish and rat retinas after injury by Western blotting analysis. In goldfish, the retinal level of Bel-2 but not Bax significantly increased 10–20 days after axotomy. By contrast, in rat the level of Bax but not Bel-2 significantly increased 6 days after axotomy. These different behaviors of Bel-2 and Bax in the goldfish and rat retina were morphologically confirmed to be limited to the RGCs by immunohistochemical analysis. The opposing properties of fish and rat retinas in cell death and survival signals offer us some clues for solving the mystery of CNS regeneration.

Keywords: axon regeneration, neurite outgrowth, regeneration, retina, visual pathway.

P14-08
Experimental crush syndrome and brain injury
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The pathogenesis of crush syndrome (CS) is severe traumatic damage to the organism accompanied by shock, stress, acute toxemia and neurodegenerative injury of brain. At experimental model of CS in Wistar rats was caused by 2 and 5 h of compression of femoral soft tissues. The electron microscopy studies carried out by us indicate that the 2 and 5 h of compression and 2, 4, 24 and 48 h decompressions increases the sizes of brain mitochondria, beginning from the small up to middle and large ones. The shape is round, and matrix is lightened. In most of M large spots of the focal lysis are observed. The outer M membrane is maintained, although it sometimes looks destroyed. The crystals lose their parallel arrangement and adopt a honeycomb-like configuration. Certain M there are transparent, and besides normal crystals honeycombs-like structures are observed. A part of M is irreversible damaged. There begins an increase of a distance between the crystals and the respiratory ensembles situated on the crystals. Their sizes increase strongly, and they are round by shape. Washing out the mitochondrial matrix takes place. Some M maintained only outer membrane with significant damages. Influence of hypothalamic cytokine – proline rich peptide (PRP, by A. Galoyan) for treatment of brain damages was investigated. Introduction of PRP on the background of 2, 4, 24 and 48 h decompression, which followed 2 and 5 h compression, is accompanied by transforming of electron microscopy views of damaged brain cells to those of intact ones. Thus, size of M decreases, the matrix is maintained in the majority of organelles, and its electron-density is higher, but in some cases the matrix is still washed out. The outer membranes is mainly maintained and it is seldom destroyed.

Keywords: brain, crush syndrome, mitochondria, proline rich peptide.
P14-09
Skeletal muscle-selective increase of glutamine synthetase gene and protein expression in ALF
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It has been suggested that skeletal muscle is responsible for the removal of excess blood-borne ammonia via glutamine synthesis, in acute liver failure (ALF). In order to further assess this hypothesis, gene expression and activities of glutamine synthetase (GS) were investigated in brain and skeletal muscle of rats with ALF because of hepatic devascularization. Arterio-venous differences for ammonia and glutamine were determined between the femoral artery and the vein. Measurement of GS activity in muscle revealed a significant increase (p < 0.01) in activity as early as 6 h following onset of ALF with further increases occurring at coma stages of encephalopathy. Western blotting and RT-PCR analysis revealed a significant increase of both, GS mRNA and protein at both precoma and coma stages. In contrast, GS activity, as well as GS mRNA and protein, were significantly reduced in cerebral cortex of ALF rats. Ammonia concentrations in the femoral vein were significantly decreased (~140 ± 5.3 mM) and venous glutamine concentrations were concomitantly increased (+0.56 ± 0.2 mM) com-
pared with corresponding concentrations in the femoral artery as early as 6 h (p < 0.01) following the onset of ALF. Thus, ALF resulting from hepatic devascularization resulted in an early sustained and selective increase in GS activity in skeletal muscle, an increase which appears to result from both pre- and post-transcriptional changes in GS. No such enzyme induction was observed in brain in ALF. These findings provide a rationale for the use of agents (such as L-ornithine-L-aspartate) with the potential to stimulate GS capacity in skeletal muscle and hence lower ammonia levels in ALF.

Keywords: brain, glutamine synthetase, hepatic encephalopathy, hyperammonemia.

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P14-10
Activation of ATP/P2 receptors and ERK signaling in strain-injured spinal cord astrocytes
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To investigate the molecular mechanisms that underlie the formation of reactive astrocytes after spinal cord injury, we have utilized a well-characterized, in vitro model of CNS trauma (1). Primary cultures of rat spinal cord astrocytes grown on deformable silastic membranes were subjected to mechanical strain (7.5 mm displacement; 50 ms), and the activity of extracellular signal regulated protein kinase (ERK), a key regulator of cellular proliferation and differentiation, was measured at various times after injury. ERK was activated about sixfold 10 min postinjury (n = 6, p < 0.001). ERK activation was blocked by U0126, an inhibitor of MEK, the upstream activator of ERK. ERK activation was partially inhibited by EGTA, indicating a role for calcium influx in strain-induced ERK activation. ERK activation was also partially inhibited by suramin and reactive blue-2, antagonists of ATP/P2 purinergic receptors, thereby indicating that trauma-induced release of ATP contributes to ERK activation. In studies with uninjured spinal cord astrocytes, we found that both P2Y, G protein-coupled receptors and P2X, ligand-gated ion channels were coupled to ERK, but further studies are needed to delineate the subtypes of ATP/P2 receptors activated after traumatic injury of spinal cord astrocytes. In summary, our results indicate that ATP released by strain-induced injury is one of the signals that triggers ERK activation and suggest a role for ATP/P2 purinergic receptors and calcium-dependent ERK signaling in the astrogliotic response to traumatic spinal cord injury.

Keywords: ATP, astrocytes, protein phosphorylation, purinergic receptors, spinal cord.

Acknowledgements: This work was supported by the Department of Veteran Affairs.

Reference

P14-11
Extensive myelin basic protein degradation in rat brain after traumatic brain injury
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Traumatic brain injury (TBI) represents a major CNS disorder without any clinically proven therapy. Axonal injury is one of the key features of TBI, yet little is known about the integrity of the myelin sheath. Here we report that the 18 kDa myelin basic protein (MBP) was extensively degraded into smaller fragments (12 and 10 kDa) in the ipsilateral hippocampus and cortex within 48 h after controlled cortical impact (a rat model of TBI). In contrast, in the contralateral counterparts, MBP was not degraded. Also, no degradation of MBP was observed in the naive and sham groups. Using proteomic-based N-terminal sequencing and tryptic digestion/mass spectrometry analysis, we have identified the exact in vivo cleavage sites on MBP after TBI. Our in vitro results also showed that MBP was sensitive to calpain proteolysis. We speculate that TBI-mediated acute neuronal cell death leads to secondary calcium influx in neighboring myelinating oligodendrocytes, which results in degradation of MBP. We further hypothesize that the MBP degradation leads to instability of the myelin sheath and initiation of demyelination, which is followed by further increases in vulnerability of exposed axons to degradation.

Keywords: axon, myelin basic protein, protease, traumatic brain injury.

P14-12
Changes of expression of chemokines after spinal cord injury
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Inflammation after spinal cord injury has led to secondary injury that participates in the difficulty in axonal regeneration. To confirm the relation between the inflammation and the difficulty in the axonal regeneration, we examined expression of chemokines, which mediates chemotaxis and activation of leukocytes and has an activity eliciting inflammation, after spinal cord transaction in the rat. Rat spinal cords were completely transected at the position of T10. The tissues adjacent to the transected site were collected respective times after injury, and also in nontransected rats the tissues were collected. Expressions of chemokine mRNAs and proteins were examined with RT-PCR and immunohistochemical analysis, respectively. mRNA expressions of monocye chemoattractant protein (MCP)-1, macrophage inflammatory protein (MIP)-1 alpha, MIP-1 beta, MIP-2, and RANTES, a family of chemokines, were remarkably increased after spinal cord transection within a few hours. The immunohistochemical staining of MCP-1 demonstrated that two kinds of cells were positive. One type of cells was motoneurons in the ventral horn, the other cells that appeared numerous at the rim of the spinal cord seemed to be macrophages or microglia. Many macrophages/microglia-like cells had a radial thin shape directing to the center of the spinal cord, and some of them adhered to MCP-1-positive motoneurons. These results demonstrate that the spinal cord transaction rapidly triggers an expression of some chemokines including MCP-1 in both motoneurons and activated macrophages/microglia around the injury site, and suggest a possible interaction between both types of cells. Chemokines may be entities that make a contact of motoneurons with activated macrophages/microglia, and result in neurotrophic and/or neurotoxic effects on motoneurons.

Keywords: chemokines, spinal cord.
P14-13
Early anti-inflammatory treatment reduces lipid peroxidation and nitrotyrosine after spinal cord injury in rats
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We have shown that blocking the interaction between the αβ2- integrin and vascular adhesion molecules for 72 h after clip-compression SCI improves neurological recovery. To investigate mechanisms for this improvement we analysed the effect of treatment with an anti-αβ2 monoclonal antibody (mAb) on myeloperoxidase activity (MPO, an indicator of neutrophil infiltration), lipid peroxidation, iNOS expression and nitrotyrosine content (indicating protein nitration) in the spinal cord after severe clip-compression injury. After the anti-αβ2 mAb treatment, the MPO activity (units/µg tissue) activity was reduced significantly from 114 ± 11 (mean ± SE) to 75 ± 8 at 6 h, from 94 ± 7 to 58 ± 10 at 24 h, from 38 ± 2 to 22 ± 4 at 72 h and finally from 9 ± 2 to 7 ± 1 at 1 week after SCI, approximating uninjured values. We quantified the amount of malondialdehyde (MDA) in the lesion area to assess lipid peroxidation. After SCI, anti-αβ2 mAb treatment significantly reduced MDA from 461 ± 57 to 342 ± 24 µM at 6 h, from 348 ± 31 to 242 ± 34 µM at 24 h and from 267 ± 16 to 163 ± 27 µM at 72 h, approximating uninjured values (125 µM). Effects of mAb treatment on the formation of iNOS and nitrotyrosine and ED-1 (an estimate of intraspinal macrophage infiltration) from 6 to 72 h after SCI were evaluated by immunohistochemistry and Western blotting. Anti-αβ2 mAb clearly reduced macrophage infiltration and attenuated spinal cord iNOS expression and protein nitration after SCI. This study suggests that beneficial effects of the anti-αβ2 mAb to block intraspinal neutrophil and macrophage infiltration reduce damage caused by reactive oxygen and nitrogen species and improve tissue preservation.

Keywords: integrin, macrophage, malondialdehyde, neutrophil, nitric oxide synthase.

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P14-14
Electroacupuncture of 2 Hz induces long-term depression of synaptic transmission in spinal dorsal horn in rats with neuropathic pain
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Objective: To observe the effect of 2 Hz electroacupuncture (EA) on long-term depression (LTD) of synaptic transmission in spinal dorsal horn in rats with neuropathic pain, so as to explore the central mechanisms of the analgesic effect of 2 Hz electroacupuncture on neuropathic pain.

Methods: In the L5/6 spinal nerves ligated neuropathic rats, the C fiber evoked field potentials in spinal dorsal horn were recorded with extracellular recording techniques. The parameters of the electroacupuncture were as follows: frequency of 2 Hz, wavelength of 0.6 ms, intensity of 1–3 mA 10 min for each intensity, stimulation time of 30 min. The positive stimulating electrode was placed in ‘sanyinjiao’ acupoint and the negative electrode in ‘zusanli’.

Results: (i) Electroacupuncture (2 Hz) significantly decreased the amplitudes of spinal C fiber evoked field potentials in neuropathic rats to 49.42 ± 0.57% of control, which lasted for at least 3 h (p < 0.001). (ii) This EA-induced-LTD of C fiber evoked potentials in spinal dorsal horn in neuropathic rats could be blocked significantly by intravenous application of NMDA receptor antagonist MK-801, or by opioid receptor antagonist naloxone.

Conclusion: EA of 2 Hz could induce long-term depression in nociceptive synaptic transmission in spinal dorsal horn in neuropathic rats. This kind of EA-induced-LTD was NMDA receptor-dependent and the endogenous opioid system may be involved.

Keywords: electroacupuncture, long-term depression, naloxone, neuropathic pain, spinal cord.

P14-15
The relationship between paraoxonase gene 192 polymorphism and atherosclerotic cerebral infarction
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Objective: To explore the relationship between the paraoxonase (PON1) gene 192 polymorphism and atherosclerotic cerebral infarction (ACI).

Method: Using polymerase chain reaction (PCR) – restriction fragment length polymorphism (RFLP), we assayed the genotype and allele frequency of PON1 gene 192 polymorphism in 52 patients with ACI and 48 healthy control subjects. Serum lipids were measured at same time.

Results: Both in ACI group and control group, the QR genotype of PON1 gene was major one, and its frequencies were 0.50, 0.54 respectively. The distribution of genotypes in the two groups was at the Hardy–Weiberg equilibriums. But the frequency of the R allele of PON1 gene in ACI group was significantly higher than that in control group (0.65 vs. 0.48, p < 0.05).

Conclusion: The PON1 gene 192 polymorphism may be associated with ACI to some extent in the chinese population. B allele of PON1 gene may be a relative risk factor in ACI.

Keywords: atherosclerosis, cerebral infarction, gene polymorphism, paraoxonase.

P14-16
Interactions between noxious visceral and vibrotactile inputs in the rat dorsal column nuclei
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Recent studies have revealed that nociceptive visceral inputs travel in the dorsal column pathway, and the activity of a given neuron in the ventrobasal thalamus can be influenced by both skin tactile and visceral nociceptive inputs (1). To determine whether interactions between the two distinct sensory modalities also took place at the lower level of the sensory pathway, extracellular single neuron recordings were carried out in the dorsal column nuclei (DCN) of anesthetized rats. Of the 237 neurons excited by vibrotactile stimuli, 31 (13.1%) units responded to colorectal distension (CRD). Amongst neurons responded to both stimuli, 23 were excited and seven were inhibited by CRD, and the remaining one was bimodal. When the vibrotactile stimuli preceded CRD, the response of neuron to CRD was reduced in comparison with CRD response alone in 13 of 30 cases. In contrast, when noxious CRD was performed before skin stimulation, the response of neuron to tactile input was often (27 of 31) enhanced. In conclusion, neurons in the DCN receive convergent tactile somatic and nociceptive visceral inputs, and interactions between the two modalities can take place at this level.

Keywords: allodynia, referred pain, somatovisceral convergence, visceral nociception.

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Reference
P14-17
The study of beta-fibrinogen gene-148C/T polymorphism and its association with cerebral infarction
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To investigate the frequency of beta-fibrinogen (Fg) gene-148C/T polymorphism and its relationship with plasma fibrinogen function and level in the patients with cerebral infarction (CI). The beta-Fg gene-148C/T polymorphism of 151 patients with CI and 113 health individual controls were analyzed by restriction fragment length polymorphism (RFLP). Fg molecular reactivity was measured by the kinetic method and Fg level was determined by turbidimetry. The frequencies of T allele in CI patients and health controls were 0.321 and 0.248, respectively (p < 0.05). The plasma Fg molecular reactivity was enhanced in cases of CI than that in health controls, 4.473 ± 0.509 and 3.716 ± 0.435 (p < 0.01), respectively. The plasma Fg level of CI patients was significantly higher than that of health controls, 3.90 ± 0.37 and 2.87 ± 0.51 (p < 0.01), respectively. The beta-Fg gene-148C/T polymorphisms are significantly associated with plasma fibrinogen levels (r = 0.52, p < 0.001) and Fg molecular reactivity (r = 0.452, p < 0.002). The beta-Fg gene-148C/T polymorphism is correlated to the plasma Fg molecular reactivity and level. The increased plasma Fg level and enhanced Fg molecular are both risk factors of CI. CI susceptibility is associated with beta-Fg gene-148C/T polymorphism.

Keywords: fibrinogen, gene polymorphism, restriction fragment length polymorphism, stroke.

P14-18
Study on the molecular mechanism of disturbed presynaptic zone in the terminals of unmyelinated afferents after peripheral axotomy
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Neuropathic pain is caused by nervous system lesions, and persists long after the initiating event has healed. Previous electron microscopic studies showed that the distribution of synaptic vesicles at presynaptic zone is disturbed in the terminals of unmyelinated afferents in lamina II of the dorsal horn of the spinal cord after sciatic nerve transection. The present study focused on the molecular basis of this abnormal distribution of synaptic vesicles. Our recent cDNA array study showed that the expression of 12 vesicle-associated genes were strongly changed in DRG after peripheral axotomy. Using in situ hybridization and RT-PCR, we confirmed the changes of 10 genes and identified the cellular distribution of six genes. Among these six genes, two genes, RAB15 and synaptoporin, were significantly upregulated in small neurons in dorsal root ganglion. These two genes may be with responsibility for the presynaptic distribution of synaptic vesicles in primary afferent terminals after peripheral axotomy. Thus, we presume that RAB15 and synaptoporin may be involved in the vesicle formation and/or trafficking.

Keywords: neuropathic pain, synaptic vesicles, vesicle-associated genes.

P14-19
Nerve injury – changes in expression and activity of matrix metalloproteinases
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One process underlying neuropathic pain is Wallerian degeneration of injured nerve fibres. Matrix metalloproteinases (MMPs) are important in this process although their expression following nerve injury remains controversial. We examined MMP-2 and MMP-9 expression after partial nerve injury. Mast cells contribute to pain responses after nerve injury. We stabilized mast cells with cromoglycate to determine whether their activation products influenced MMP expression. The left sciatic nerve was partially ligated in male Wistar rats anaesthetized with pentobarbitone sodium and halothane/O2 (50 of 50). Gelatin zymography was used to detect latent and active forms of MMP-2 and MMP-9; positive bands were semiquantitated by image analysis. In normal nerve, the latent and active forms of MMP-2 were consistently expressed at a high level, whereas the activity of MMP-9 was just detectable. Contrary to previous reports, nerve injury reduced levels of pro-MMP-2 by 18% 12 h postinjjury. Active MMP-2 was reduced by 36% at 12 h and 24% at 3 days. Following nerve injury, substantial pro-MMP-9 and some active MMP-9 were detected at 12 h and 3 days after nerve injury. Interestingly, MMP-9 was also found in the contralateral nerve, although activity was ~30% less than that in the injured nerve. Expression levels of MMP-2 and MMP-9 were similar in the mast cell-stabilized group. Our findings indicate that the balance of MMP-2 and MMP-9 is altered after nerve injury. The significance of decreased MMP-2 activity following nerve injury remains to be elucidated.

Keywords: injury, mast cell, metalloproteinases, nerve.

P14-20
Identification of a novel FERM domain-containing gene whose expression is induced after optic nerve injury
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Axons of adult mammalian central nervous system usually do not regenerate after injury. When the axons of retinal ganglion cells are damaged by trauma or accident, however, there is an abortive regeneration, suggesting retinal ganglion cells may have limited ability to regrow axons. To find out the factors that may augment neuronal regeneration, we had previously adopted subtractive hybridization method to search for genes whose expression is up-regulated after optic nerve injury. One DNA fragment, temporarily named 9a8, was identified in this analysis and was verified by semiquantitative RT-PCR to be a gene whose expression increases after the optic nerve injure. Upon further cloning, it was revealed that this gene encodes an open reading frame of 570 amino acids and contains a FERM domain at N-terminus. The molecular weight of this protein is about 65 kDa as shown in Western blot. Strong expression of 9a8 is in retina, brain, and heart of adult mice. 9a8 protein is detected in retinal ganglion cell layer, outer plexiform layer, inner segment of photoreceptor, and retinal pigment epithelium by immunohistochemistry. Indirect immunofluorescent staining of HEK293T cell line with monoclonal antibody has identified that 9a8 protein was present in the cytoplasm. Whether the expression of 9a8 in protein level is up-regulated after optic nerve injury is currently under investigation.

Keywords: axon regeneration, FERM domain, neurotrauma, optic nerve, retina.
P14-21
Induction of retinal telomerase activity during optic nerve regeneration after optic nerve cut
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Telomerase is an enzyme stabilizes chromosomes by adding telomeric repeats (TTAGGG) to chromosomal ends. Telomerase activities are commonly found in cancers and organs with high turnover rate. The aim of this study was to investigate the regulation activity of telomerase in retina during retinal ganglion cell regeneration. Right optic nerve cut was performed in 9 weeks old Sprague-Dawley rats. Peripheral nerve graft and rat ciliary neurotrophic factor (rCNTF) injection were performed immediately after nerve cut to facilitate optic nerve regeneration. Animals in control groups received only optic nerve cut but no graft or injection. Groups of animals were serially sacrificed 1, 3, 7, 14, 21 and 28 days after operation. Retinas of the animals were dissected for telomeric repeat amplification protocol (TRAP) assay followed by densitometric quantification. Relative abundance of telomerase in different layers of retina was examined by immunohistochemistry (IHC) method. The expression levels of telomerase mRNA were assessed by RT-PCR. The telomerase activity in retinas increased significantly during nerve regeneration. The activity reached a maximum and then decreased to nearly normal level. The results show that telomerase activity is regulated during nerve regeneration. Further studies on the mechanism of telomerase regulation during nerve regeneration are required to understand the role of telomerase in central nervous system regeneration.

Keywords: nerve regeneration, optic nerve cut, telomerase, telomeric repeat amplification protocol assay.

P14-22
Tumour necrosis factor-alpha induces beta-adrenergic receptor expression in cultured primary rat astrocytes
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It is known that the levels of tumour necrosis factor-α (TNF) and glial fibrillary acid protein were increased following brain injury, and that β-adrenergic receptor (β-AR) antagonists suppressed astrogliosis. Based on the above findings, we hypothesized that TNF and β-adrenergic mechanism may interact in astrocytes. Among the cytokines tested, TNF was found to be most effective in inducing astrocyte proliferation, while interleukin-6 was almost ineffective. TNF induced the expression of TNF, TNF receptor 2 (TNFR2), as well as b1- and b2-AR as revealed by RT-PCR. These inductions were suppressed by prior treatment with selective TNFR2 antibody. Furthermore, the TNF induction was not blocked by H89, a potent protein kinase (PK) A inhibitor, but reduced by selective PKC inhibitors: staurosporine and Ro 31-8220. Collectively, our findings suggest that TNF induces genes expression in cultured astrocytes by interacting with TNFR2 and the signaling pathway involved is likely to be PKC-dependent. Moreover, this study suggests TNF action and β-adrenergic mechanism are closely related in cultured astrocytes.

Keywords: astrocytes, β-adrenergic receptor, PKC, tumour necrosis factor-α.

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P14-23
PYK2 binding to tank constitutes an intra-axonal sensor for inflammatory stimuli after optic nerve crush
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Proline-rich tyrosine kinase 2 (PYK2) was identified as an upregulated transcript in a screen for differentially expressed genes in retinal ganglion cells (RGC) after optic nerve crush. PYK2 is highly phosphorylated after axonal injury with increased axoplasmic immuno-reactivity in apposition to the crush site. Autophosphorylation of PYK2 is regulated by tumor necrosis factor-α (TNF-α), which is synthesized from infiltrating macrophages in the crushed nerve. Interestingly, blockade of both, TNF-α signaling or the optic nerve inflammatory response after crush attenuated phosphorylation of PYK2. Anterograde transport of TNF-R1 receptor, the TRAF-associated NF-xB activator (TANK) and PYK2 and co-immunoprecipitation of TANK and PYK2 suggest a coupling of PYK2 to an axonal TNF-R1 receptor. PYK2 is directly interacting with TANK, a coinducer with TRAF2 of NF-xB activation. Yeast two-hybrid studies locate the PYK2 binding site at the N-terminal part of TANK. Mutation of the PYK2 autophosphorylation site, Tyr402, which is essential for PYK2 activity, reduces the TNF-α activation of NF-xB. Inhibition of PYK2 autophosphorylation reduces the number of surviving retinal ganglion cells and nuclear NF-xB accumulation after optic nerve crush. Thus, PYK2 seems to be an axoplasmic sensor coupling the inflammatory status of the optic nerve to a retrograde survival promoting signaling pathway from the injured axonal compartment to the nucleus.

Keywords: axon, axon regeneration, neurodegeneration, neuroimmune modulation, neurotrauma.

P14-24
Gene expression profile reveals major changed genes in the rat spinal dorsal horn after peripheral axotomy
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Spinal dorsal horn plays an important role in pain transmission and modulation. In the previous study, we made the cDNA microarray membranes by using the clones from dorsal root ganglion libraries and compared the gene expression profile between normal and axotomized DRG neuron. In this paper, we further study the gene expression in dorsal horn after peripheral axotomy in order to identify the genes participate in pain transmission and modulation in second neurons. Totally 184 known genes and 66 ESTs or novel ESTs were obtained by cDNA microarray analysis. Twenty-six genes or ESTs were confirmed by RT-PCR and/or Northern blotting and/or immunohistochemistry. Those known genes were divided into seven classes according to their functions and structures. We find that ion channels, receptors and signal transduction modulators and effectors were changed markedly in the dorsal horn after peripheral axotomy, especially some kinases such as several members of the mitogen-activated protein kinase (MAPK) superfamily and some protein kinase C (PKC) subtypes were up-regulated in the dorsal horn after axotomy, implying their potential roles in pain transmission and modulation. Most interesting several drug target genes such as GABAA receptor α5 subunit, t-type calcium channel α2δ1 subunit for gaba-pentin were up-regulated in dorsal horn after peripheral axotomy, indicated applying drugs in spinal cord also a useful tool for treatment certain type of neuropathic pain.

Keywords: gene expression, pain, spinal cord.
**P14-25**

**Investigation of ependymal functions in health and disease by analysis of ependyma-specific gene expression**

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The ependyma is a single-layered, mostly ciliated epithelium at the interface between the parenchyma of the central nervous system and the ventricular space. The expression in these cells of aquaporins and receptors for natriuretic peptides suggest a role for them in the control of water fluxes in the brain and the CSF. These cells have therefore been implicated to be involved in the pathogenesis of post-traumatic brain edema. Since no concise evidence on this matter has yet been obtained, an effort was made to find genes specifically expressed in this cell type. The specific gene expression of the ventricular epithelium was analyzed by screening a subtractive cDNA library from bovine brain, constructed by the suppression PCR method, in which ependymal RNA was used as tester and ependymal-free brain RNA as driver. Several clones were identified, which show no expression in heart, liver, kidney, spleen and small intestine, weak expression in brain and strong expression in ependymal primary cultures, lung and testis, as detected by RT-PCR analysis. Database analysis revealed one of these clones to represent sperm-associated antigen 6, a protein of the ciliary and flagellar axoneme apparatus. The distinctive expression profile suggests that some of the other cDNA sequences in question also code for constituents of the axoneme apparatus, because only these tissues contain kinocilia. Antibodies are being generated against peptides derived from the cDNA sequences of these clones to confirm the ciliary location of the proteins. Antibodies already generated against another cDNA-derived peptide suggest a role of the corresponding protein not connected with cilia but possibly with secretion.

**Keywords:** complementary DNA, DNA library, gene expression, glial cells, immunocytochemistry.

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**P14-26**

**Carpal tunnel syndrome – confirmation of diagnosis in clinically positive subjects with nerve conduction studies**

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Slowing of nerve conduction in carpal tunnel syndrome (CTS) occurs in the palm to wrist segment of the hand. CTS is diagnosed by comparing median and ulnar nerve conduction velocity and latency. The objectives of the study were to assess the motor and sensory conduction of median and ulnar nerves in patients with symptoms suggestive of CTS and determine the clinical use of nerve conduction in CTS. In a cross-sectional study, adult patients (n = 72) with symptoms of CTS were studied. Of 121 (84%) hands of patients presenting with suggestive symptoms were diagnosed of CTS. The median nerve motor conduction velocity (M-VCV) was statistically significantly decreased when compared with the ulnar nerve motor conduction velocity (U-VCV) in patients with mild (p = 0.0001) and severe CTS (p = 0.00001). The median nerve sensory conduction velocity (M-SCV) was significantly decreased when compared with the ulnar nerve sensory conduction velocity (U-SCV) in severe CTS (p = 0.006). The median nerve motor latency (M-ML) was significantly decreased when compared with the ulnar nerve motor latency (U-ML) in mild (p = 0.0001) and severe CTS (p = 0.00001). The median nerve sensory latency (M-SL) was significantly decreased in severe CTS (p = 0.0001). Comparison of median and ulnar, motor and sensory conduction velocities allowed for the determination of abnormalities in 121 hands (84%) and yielded improved diagnostic rate compared with M-SCV [48 (40%) alone, M-ML [92 (76%)] is more valuable than M-SCV [48 (40%)] and M-SL [35 (29%)] for diagnosis of CTS. In patients suspected of CTS, studying motor and sensory conduction of median nerve and comparing with ulnar nerve conduction improved the diagnostic yield.

**Keywords:** degeneration, motor neurons, peripheral nerve, sensory neurons.

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**P14-27**

**Over-express endothelin-1 in astrocyte lead to increased brain damage and altered cytokine expression after cerebral ischemia**

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Previously, we reported that the expression of endothelin (ET-1) is induced in astrocytes and endothelial cells after cerebral hypoxia/ischemia. Recently, we also showed that transgenic mice over-expressing ET-1 in astrocytes displayed more severe neurologic deficits and increased brain infarct following transient middle cerebral artery occlusion (MCAO for 2 h followed by 22 h reperfusion) compared with those of non-transgenic mice. However, the underlining mechanism for further brain damage in these transgenic mice with astrocytic ET-1 over-expression is not yet clear. ET-1 has been shown to stimulate leukotriene (LTB4) and cytokine expression such as interleukin 6 (IL-6) after MCAO, which may contribute to brain inflammation and damage. Here, we investigated cytokine expression in brain of astrocytic ET-1 transgenic mice to determine whether the altered cytokine expressions may have lead to further brain damage in these transgenic mice. Group of transgenic and non-transgenic mice were exposed to either sham or transient MCAO. Cytokine mRNAs from these mouse brains were detected by using the RiboQuant multi-Probe Ribonuclease Protection Assay system. The brain of astrocytic ET-1 transgenic mice treated with the transient MCAO showed increased level of macrophage migration inhibitory factor (MIF), whereas IL-6 level did not show any changes. The present data suggest that altered expression of MIF in the brain of astrocytic ET-1 transgenic mice may have contributed to more severe neurologic deficits and increased brain damage after MCAO.

**Keywords:** astrocytes, cytokines, endothelin, ischemia, transgenic mouse.

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**P14-28**

**Is carpal tunnel syndrome related to gender, age and body mass index? A Sri lankan study**

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The study aimed to investigate the variation in sensory and motor nerve conduction to age, gender and body mass index (BMI) amongst subjects with symptoms suggestive of carpal tunnel syndrome (CTS). Seventy-two adults with suggestive symptoms were studied. Sensory (SCV) and motor nerve conduction (MVC) velocities were measured in median and ulnar nerves of both upper limbs. Subjects who fulfilled two or more of the diagnostic criteria were confirmed of CTS. The diagnostic criteria set were (a) difference in sensory latencies between median and ulnar nerves >0.05 ms, (b) distal median nerve motor latency to Abductor pollicis brevis muscle >4.2 ms, and (c) median nerve motor conduction velocity <48 ms. CTS was confirmed in 121 (84%) limbs and were categorized into absent CTS [23 (16%)], early CTS [50 (41%)] and CTS [71 (49%)]. The male:female ratio in the study group was 1:4.5. The mean BMI and age were not significantly different in the CTS group when compared with the non-CTS group (p > 0.05) and early CTS group (p > 0.05). The mean BMI was significantly negatively correlated to the median motor conduction velocity in the early CTS group and the CTS group. The mean BMI, and age were not significantly higher in the early CTS group and the CTS group, unlike in other studies of the world. This may be because of low or borderline nutritional status in the Sri Lankan population. CTS occurred mostly among the females as in other studies of the world.

**Keywords:** aging, degeneration, peripheral nerve.
P14-29
Early, selective loss of GLT-1β in the cerebral cortex following lateral fluid-percussion injury
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Glutamate transporter proteins are essential for the control of extracellular glutamate levels, with an impairment of their activity or gene expression being a major potential contributor to excitotoxicity. Lateral fluid-percussion (LFP), a clinically relevant model of traumatic brain injury (TBI), leads to neuronal cell death. In this study, we have investigated the effects of LFP on the astrocytic glutamate transporters GLT-1α, GLAST, and the splice variant GLT-1β, as well as the neuronal glutamate transporter EAAC-1 in order to evaluate their role in the sequelae of events underlying trauma-induced injury. Male Sprague-Dawley rats (375–400 g) were subjected to moderate trauma (2.0–2.5 atm.) and normalized Western blotting techniques combined with immunohistochemistry were used to investigate changes in these transporters in the injured cerebral cortex of rats at 6 and 24 h post-TBI. Although GLT-1α, GLAST and EAAC-1 protein content remained unchanged compared with shams at these times, levels of GLT-1β were decreased by 67% as early as 6 h post-TBI, and were sustained for up to 24 h following injury. This effect was accompanied by a transient 67% decrease in GFAP content at 6 h following trauma and a 76% reduction in the levels of the neurofilament protein α-internexin. These results suggest that LFP leads to a selective loss of GLT-1β that is not because of astrocytic cell death, but which is concomitant with neuronal damage. Selective down-regulation of this glutamate transporter splice variant may play an important role in the pathophysiology of trauma-induced brain injury.

Keywords: excitatory amino acid, glutamate transporter, trauma.

P14-30
The cellular cytokine, IL-6 is upregulated in response to cAMP and can abrogate the MAG and myelin-mediated block of axonal regeneration in vitro
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The lack of axonal regeneration observed following injury to the adult mammalian CNS is attributable to several factors, including the myelin-associated inhibitors. Recent evidence has shown that increasing intracellular cAMP levels can abrogate the inhibitory effects of these molecules in a transcription-dependent manner. The precise genes that are upregulated in response to cAMP elevation, however, still remain a mystery. Results from both microarray analysis and quantitative RT-PCR show that there is a robust increase in expression of the cytokine, IL-6. Furthermore, when added to primary neurons in culture, IL-6 can overcome the inhibition of axonal growth by both MAG and myelin in a dose-dependent and transcription-dependent manner. In addition, following intrathecal delivery of IL-6 via mini-osmotic pumps, DRG neurons are able to extend long neurites when subsequently grown on a monolayer of MAG-expressing CHO cells. Finally, blocking of the IL-6-induced signaling elements gp130 or JAK, can abrogate the IL-6-induced reversal of the MAG and myelin-mediated inhibition of neurite outgrowth. Taken together, this data suggests that IL-6 may be one of the regeneration-associated genes, which play a role in the cAMP-induced enhancement of axonal regeneration following CNS injury.

Keywords: CNS, axonal regeneration.