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SPEAKER ABSTRACTS
(Fully published articles have been removed from this section)

K-1

Changing concepts in brain energy metabolism

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Our understanding of brain metabolism and neuronal-astrocytic interactions are continuously changing and evolving as better tools become available for unraveling the metabolic specialization(s) and interactions of brain cells. Techniques including improved microscopy, cell isolation techniques, targeting of specific tagged/inducible genes and fluorescent proteins to specific organelles such as mitochondria in astrocytes and neurons, *in vitro* and *vivo* imaging of Ca^{2+} fluxes and NADH formation in cells and brain and 13C-NMR spectroscopy have provided new information and insights that have changed our understanding of brain metabolism over the past two decades. Brain energy metabolism remains an exciting, constantly changing and clinically relevant field. As investigators we must rigorously evaluate new findings and be willing to embrace new concepts when there is solid evidence that the concepts are valid for metabolism *in vivo*. We must also maintain a healthy skepticism regarding concepts for which the evidence is exclusively from cell culture studies and not yet demonstrated or confirmed *in vivo*. It is crucial to identify important areas where more studies, and perhaps different approaches, are needed to increase our knowledge and understanding of the dynamics of metabolic regulation and inter- and intracellular trafficking and compartmentation of metabolism in brain. Several changing concepts and areas of controversy will be presented.

K-2

Astrocytic purines regulate sleep homeostasis and memory loss following sleep deprivation

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Using inducible astrocyte-specific transgenic mice we have investigated the role of astrocytes in the control of synaptic transmission, neuronal networks and behavior. When a dominant negative SNARE domain is selectively expressed in astrocytes the extracellular level of adenosine responsible for the activation of A1 receptors is reduced. As a consequence we find an enhancement of excitatory synaptic transmission. Adenosine and A1 receptor activation has been proposed to play important roles in the regulation of sleep homeostasis. Using EEG and EMG recordings from freely behaving dnSNARE mice we find that sleep homeostasis is impaired: following sleep deprivation the compensatory increase in total sleep time is abolished which is mimicked in wildtype mice by infusion of the A1 receptor antagonist CPT. It is known that sleep loss leads to impairments in memory formation. When dnSNARE is expressed in astrocytes sleep deprivation induced impairments in memory formation are prevented. Taken together these results provide the first demonstration of roles for astrocytes and gliotransmission in behavior.

S-03

Regulation of expression and distribution of monocarboxylate transporters in neurons and glia

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Monocarboxylate transporters (MCTs) are proton-linked membrane carriers involved in the transport of lactate, pyruvate, as well as the ketone bodies acetoacetate and β-hydroxybutyrate. MCT1, MCT2 and MCT4 are three members of this large family that have been found to be expressed in the central nervous system. MCT1 is strongly expressed by endothelial cells forming blood vessels as well as by astrocytes. MCT2 is the predominant neuronal monocarboxylate transporter while MCT4 is exclusively found on astrocytes. A series of experiments involving MCT overexpression/downregulation in either neurons or astrocytes in culture has provided evidence for the participation of specific MCTs in lactate utilization or release by each cell type. However, little information is available about regulation of MCT expression in either physiological or pathophysiological situations. We have recently shown a strong enhancement of MCT expression in the brain of obese mice. Neurons in specific areas exhibited enhanced expression not only of MCT2 but also MCT1 as well as MCT4. Similar changes were observed in *ob/ob* and *db/db* mice, two models of obesity that are characterized by hyperglycemia and hyperinsulinemia. Investigations in cultured neurons revealed that insulin induces the expression of MCT2 by a mechanism involving a stimulation of translation. In addition to insulin, noradrenaline as well as IGF-1 have been shown to enhance MCT2 expression in neurons by a similar translational mechanism. More specifically, it was shown that all three substances activated the PI3K/Akt/mTOR/S6K pathway. Further experiments suggest that subcellular distribution of MCT2 can be also modulated by neuroactive substances. It appears that MCT2 can be either translocated to the plasma membrane or rather internalized into an intracellular pool. Thus, it appears that MCT expression and distribution can be modulated in specific cell types upon various conditions and by different mechanisms. In particular, it is suggested that such changes might allow to adjusting energy substrate supply to varying levels of synaptic activity.

S-05

Neural function of glycogen

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The brain, muscle and liver isoforms of glycogen phosphorylase (GP BB, GP MM, and GP LL) are the lead enzymes for the cellular presence of glycogen metabolism. In brain and spinal cord GP BB is found in all astrocytes and ependymal cells and a few neurons. Astrocytes, in addition, contain also GP MM. The brain isoform can be considered a marker for the peripheral nervous system (PNS) since this is the sole GP isoform found in somata and processes of all peripheral neurons. The glycogen of neural cells can generally serve as the source of most rapidly mobilizable glucosyl residues, e.g. (i) for the glycolytic production of the standard fuel molecule lactic acid that

is forwarded by astrocytes to adjacent neurons; (ii) for the generation in the pentose phosphate pathway of the NADPH needed for the detoxification of reactive oxygen species; (iii) for the energization of nerve fibers and their presynaptic terminals of PNS neurons.

S-07

New approaches to and insights into glucose, glycogen, glutamate and glutamine metabolism in brain

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NMR is a unique investigative tool for studying compartmentalized neuronal glial metabolism. We have recently introduced a novel approach to modeling TCA cycle flux for the measurement of oxygen metabolism and have extended this approach to the measurement of acetate metabolism in the brain to ascertain glial metabolis fluxes, as well as expanded it to the modeling of ^{11}C-acetate positron decay curves. Along a second line of experimentation we have established a novel protocol suitable for quantifying glial glycogen content in the brain using NMR and ^{13}C labeling techniques, using a prelabeling protocol consisting of 24 h of ^{13}C glucose administration. As part of establishing this protocol, which requires the assessment of ^{13}C enrichment of glycogen, which was validated to be possible from that of NAA C6, we developed a novel selective editing method, allowing to measure NAA turnover in the presence of a highly labeled overlapping Glx C3 resonance even when using indirect detection. The sensitivity of ^{13}C studies can be augmented by using DNP to generate hyperpolarized ^{13}C acetate, which can be detected in the rat brain *in vivo* using a home-built polarizer setup, provided T1 is sufficiently long (approximately 10 s). Finally we have extended the detection of the neurochemical profile to full sensitivity acquisition at very short echo times (approximately 3 ms), which enabled the detection of the neurochemical profile, including glucose, on a 14T/26cm bore magnet showing changes in Gln/Glu ratio following ischemia, consistent with exctitoxic damage from glutamate release, as well as a more pronounced reduction of NAA (as well as Tau) than Glu, suggesting the former is a sensitive marker of neuronal integrity. Finally, glucose concentrations remained elevated despite the post-ischemic presence of lactate, implying that the elevated lactate is not accompanied by a drastic increase in anaerobic glycolysis. Finally, in experimental diabetes, glucose transport was unchanged and Tau and myo-inositol show changes suggesting that these two compounds are the primary idiogenic osmolytes in brain. In summary, using NMR spectroscopy neurochemistry and brain energy metabolism of many important metabolic processes can be investigated *in vivo*, allowing elucidating the mechanisms of neuro-glial interaction in health and disease models.

S-09

New approaches for metabolic modeling of ^{13}C NMR data using two-compartment neuronal-glial models

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Carbon-13 NMR spectroscopy has great potential to measure quantitative metabolic rates in the brain *in vivo.* In particular, measurement of glutamate and glutamine ^{13}C turnover curves during [1-^{13}C] or [1,6-$^{13}C_2$] glucose infusion and subsequent metabolic modeling with two-compartment neuronal-glial models has allowed non-invasive measurement of compartmentalized metabolic rates such as the glial and neuronal TCA cycle rates, the rate of pyruvate carboxylase, and the rate of the glutamate-glutamine cycle (V_{NT}) between neurons and astrocytes, which has been proposed to reflect glutamatergic neurotransmission. However the reliability of complex two-compartment metabolic models has not been investigated systematically until recently. A recent study performed using Monte-Carlo simulations with a previously published metabolic model suggested that the determination of glial TCA cycle rate and the rate of glutamate–glutamine cycle is not very precise when using [1,6-$^{13}C_2$] glucose as the infused substrate. Additional simulations suggest that including a glutamine isotopic dilution flux in the model leads to better determination of V_{NT} (not shown). Therefore, even small modifications in the metabolic model may strongly affect how reliably metabolic fluxes can be determined. This is an important observation because two-compartment models have been evolving constantly over the past 10 years. For example, initial metabolic models did not include independent parameters for glial TCA cycle flux, pyruvate carboxylase flux, or glutamine dilution flux. In this presentation, we will present our latest Monte-Carlo simulations results and explore new strategies to make two-compartment metabolic modeling more precise and reliable. One strategy is to use acetate as a substrate instead of glucose. Acetate is exclusively taken up by glial cells and therefore may lead to better determination fluxes related to glial processes. A second strategy is to infuse multiple substrates simultaneously, for example co-infusion of glucose and acetate. A third strategy is to take advantage of the additional information available in ^{13}C isotopomers, which appear as multiplets in ^{13}C spectra. This information is typically not used in current metabolic models of brain metabolism. We will present results of Monte-Carlo simulations to assess the precision of metabolic modeling with each of these strategies, as well ^{13}C MRS data measured *in vivo*. In conclusion, we expect that new methods that we are developing to perform metabolic modeling of ^{13}C NMR data using two-compartment neuronal-glial models will lead to improved precision on the determination of metabolic rates as is currently possible.

S-11

The astrocytic networks participate in the functional hyperemia via metabolic waves induced and sustained by extracellular glutamate

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The blood flow increases locally in the cerebral cortex as a response to changes in the neuronal activity, a phenomenon named functional hyperemia. Several recent findings suggest that the initial component in this phenomenon could be triggered directly by both excitatory and inhibitory specialized neurons. In contrast, Ca^{2+}-mediated signaling inside the gap-connected astrocytic network, induced by extracellular glutamate, may take part in a more robust vascular response to meet long term metabolic needs. This astrocytic pathway has been suggested to lie beneath a vascular *tonic modulation*. The physiological mechanisms and biochemical triggers underlying this pathway remain unclear today. In this study, we observed non-concurrent changes in local field potentials (LFP),

multi-unitary activity (MUA) and astrocytic Ca^{2+} concentrations produced by whisker stimulation at a wide range of physiological conditions. The whiskers were stimulated by using 10ms air-puffs at three frequencies (1, 3 and 5 Hz). We compared the time course of these two signals for dissimilar stimulus durations (2, 8, 32, 128 s). A craniotomy of 2 mm in diameter was performed on the barrel cortex of five P21 Wistar rats (50–70 g). LFP/MUA recordings were observed by using silicon-substrate probes (1D-shank, silicon dioxide/nitride insulation, 16 linearly arranged iridium electrodes) connected to a 50 kHz amplifier and a processing unit (PZ2/RZ2, TDT). Ca^{2+} concentrations were observed by using a calcium-indicator dye (Fluo-4, AM ester) through a two-photon laser scanning microscope (FV1000-MPE, Olympus, Tokyo, Japan), with a customized *in vivo* stage for small rodents. Astrocytes were labeled with sulforhodamine 101. A cocktail containing the fluorescent dyes were applied either directly to the exposed cortical surface or by using micro-injection through a micropipette (5–9 MΩ). For two-photon imaging, the exposed cortex was sealed with warmed agarose and a cover glass was used to reduce imaging artifacts produced by vasomotions. We analyzed the correlation between the neuronal activity and the astrocytic Ca^{2+} signaling, which must be a good sign for the existence of a multi-cellular crosstalk. We conclude that depending on the time scale for the neuronal events, at least two pathways are involved in the functional hyperemia.

S-13

GABA synthesis and glial function: insights from NMR studies of rats and mutant mice

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Glutamatergic and GABAergic neurons represent the majority of neocortical neurons. The contribution these neurons make to overall glucose oxidative metabolism in the cerebral cortex and how their neurotransmitter fluxes relate to changes in activity are uncertain. Using nuclear magnetic resonance (NMR) spectroscopy with infusions of [1, 6-^{13}C] glucose, which is metabolized by neurons and glia, and the glial specific substrate, [2-^{13}C] acetate, glutamatergic and GABAergic fluxes can be separately determined. These studies have shown that GABA metabolism contributes approximately 20% of the total glucose oxidized by glutamate and GABA neurons and a similar percentage of total (glutamate-GABA/glutamine) cycling. To explore the role of the GAD isoforms in GABA synthesis we took advantage of the sensitivity of GAD67 protein to elevations in intraneuronal GABA concentration (GAD67 is reduced as GABA levels rise), the later produced by treatment of rats with a GABA-transaminase inhibitor. GAD65 is unaltered by this treatment. Rats depleated in GAD67 were studied under basal conditions and during strong cortical activation (seizure) induced by bicuculline. We found that a reduction in GAD67 correlated with a substantial loss in basal synthesis but that stimulated GABA synthesis (which could be attributed to GAD65) was unaltered. The findings are consistent with the idea that GAD67 and GAD65 mediate synthesis of different GABA pools, e.g., cytoplasmic and vesicular. To gain additional insight into GABA catabolism, and its potential importance in the astroglia, we studied mice deficient in succinic semialdehyde dehydrogenase (SSADH), the second of two enzymes involved in the degradation of GABA to succinate. SSADH deficient mice have high concentrations of GABA and gamma-hydroxybutyrate (GHB), a product of increased succinic semialdehyde, and develop status epilepticus during the critical postnatal period (P17P20) of development. SSADH null mice infused with [1, 6-^{13}C] glucose or [2-^{13}C] acetate revealed significant reductions in glutamate and glutamine labeling from these precursors, possibly reflecting reduced metabolism in neurons (glutamatergic) and trafficking to these neurons of glial glutamine. Surprisingly, rates of GABA and GHB synthesis were unaltered, possibly supported by siphoning (and eventual trapping) of available glial-derived precursors.

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S-18

Human brain metabolism studied by ^{1}H NMR spectroscopy

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Methodologies of proton magnetic resonance spectroscopy (^{1}H MRS) allow the non-invasive measurement of metabolite concentrations in the human brain, along with their changes induced by variations in neurotransmission activity. This capability provides unique and valuable information to understand the metabolism of the brain at work. In fact, changes of metabolite concentrations during activation imply changes in metabolic patterns, such as preferential use of different pathways or alteration of fluxes, even though changes in metabolic fluxes can be accomplished without changes in metabolite concentrations. The reliable quantification of changes in metabolite concentrations during studies of functional ^{1}H MRS is a demanding task, and indeed studies reported in literature have often produced inconsistent results. Ultra-high magnetic field systems can help in obtaining robust and accurate time-courses of metabolites. The concentrations of 17 brain metabolites were recently measured with unprecedented sensitivity and temporal resolution at 7 T in the human primary visual cortex during paradigms of visual stimulation. However, in spite of the increased reliability for metabolite quantification attainable at ultra-high fields, the temporal and spatial resolution of *in vivo* ^{1}H MRS is not suitable to provide direct evidence to prove or disprove those hypotheses that involve metabolic compartmentalization between different cell types, because the measured signal is averaged from relatively big area of the cortex. Importantly, *in vivo* ^{1}H MRS cannot provide direct measurements of the amounts of lactate and glucose that are specifically used by neurons and astrocytes during activation, and the interpretation of changes of lactate concentration ([Lac]) *in vivo* is anything but straightforward. Therefore, the analysis of tissue [Lac] changes in the context of compartmentalized metabolism requires the formulation of mathematical models, a goal that has been tackled by other groups Aubert and Costalat. This talk aims at describing how the current hypotheses of brain metabolism relate to

the experimental findings obtained *in vivo* by ^{1}H MRS regarding functional changes of metabolite concentrations. Specifically, the contribution of anaerobic and aerobic processes will be discussed. Then, changes in tissue [Lac] measured *in vivo* during visual stimulation will be considered within the framework of the models presented by either Aubert *et al*. or by Simpson *et al.* Finally, new results will be presented regarding innovative developments of ^{1}H MRS to investigate metabolite dynamics *in vivo*. Such applications do not rely on the measurement of metabolite concentrations, but rather on the estimation of their fundamental NMR relaxation parameters (i.e., intrinsic relaxation rate constants, populations and the exchange rate constants between different sites) during an application of adiabatic pulses.

S-20

Spatio-temporal characteristics of exocytosis in astrocytes

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The mechanism underlying Ca2+-dependent release of various transmitters from astrocytes is exocytosis. Astrocytes express the protein components of the SNARE complex, including synaptobrevin 2, syntaxin and SNAP-23, but not SNAP-25. Using astrocytes expressing synapto-pHluorin, exoctytotic sites can be fluorescently imaged. Solitary astrocytes predominantly exhibit exocytotic fusion sites at the plasma membrane in the perinuclear region of the astrocytes, while astrocytes in contact with other cells show their fusion sites evenly distributed between the central and peripheral (location of cell-cell contact) regions; this contact-directed distribution of fusion sites is regulated by the gap junction protein, connexin 43. Fusions of synapto-pHluorin labeled vesicles with the plasma membrane can be observed using total internal reflection fluorescence microscopy; the time course of fusion events (burst vs. sustained), their type ('kiss-and-run' vs. full fusion) and spatial relationship between different fusion sites is discussed. Single molecule investigations of the SNARE complex using force spectroscopy show that ternary complexes containing SNAP-23 have a shorter spontaneous lifetime than those containing SNAP-25B. Thus, the spatio-temporal characteristics of astrocytic exocytosis might be in part due to intrinsic properties of the ternary SNARE complex in astrocytes.

S-21

Dynamin inhibitors reduce synaptic transmission and are novel anti-epileptic drugs

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Typically a nerve terminal contains about 200 synaptic vesicles (SVs) which rapidly recycled to prevent their depletion, otherwise synaptic fatigue occurs. SV endocytosis (SVE) retrieves them after exocytosis. The protein dynamin plays a crucial role at the final step in SVE. Dynamin has the ability to bind, manipulate and fragment lipid by assembly into rings and helices around the necks of budding vesicles. An assembly-dependent burst of GTPase activity mediates membrane fission. Dynamin-dependent endocytosis is a rate-limiting step for synaptic transmission because it controls the supply of SVs. It becomes rate-limiting under conditions of moderate or prolonged stimulation. Thus incubating synaptosomes with a phospho-mimetic peptide that blocks syndapin binding to dynamin inhibits SVE. The peptide causes a rundown of glutamate release when stimulation is maintained for longer than 45 s. This has clinical implications for treating neurological disorders such as epilepsy. Epilepsy is a disease of synaptic transmission affecting up to 1% of the population. Seizures may be managed by anti-epileptic drugs, but over 30% of people don't respond to any drugs. Targeting the GTPase activity of dynamin I is an attractive candidate for a novel anti-epileptic drug as it would not affect synaptic transmission at lower frequency, but would reduce synaptic transmission during excessive brain activity. We have developed a range of drugs that inhibit dynamin by four distinct mechanisms. The PH domain is targeted by several series of long chain amines based on myristoyl trimethyl ammonium bromide (MiTMAB). Dynamin assembly is targeted by a large series of Bis-T and dyngo analogues. The GTPase domain is targeted by indole analogues. Over 19 other series of drugs are under development. The drugs reversibly inhibit SVE in cultured neurons and cause an activity-dependent run-down in synaptic transmission. Representatives of the best drugs in each class have anti-convulsant activity in animal models at the NIH. These studies illustrate how revealing the details of the molecular mechanisms of synaptic transmission may ultimately lead to new therapeutic approaches for treatment of disorders of synaptic transmission.

S-24

Glutathione, mitochondrial function and neurodegeneration

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The mitochondrial electron transport chain is particularly vulnerable to oxidative inactivation. When considering brain cells, evaluation of cultured neurones and astrocytes reveal an apparent differential susceptibility to reactive nitrogen species such as peroxynitrite. Thus, neuronal cells appear particularly vulnerable with a loss of mitochondrial respiratory chain activity, at the level of complex IV (cytochrome oxidase) and, with increasing concentrations, cell death. In contrast, astrocytes appear to be able to resist comparable oxidant exposure and maintain their cellular energy status, despite loss of mitochondrial function, by up-regulating flux through glycolysis. In addition, the differential susceptibility of these cells, when cultured alone, appears to be related to glutathione (GSH) metabolism, i.e. GSH status of astrocytes is superior to that of neurones and oxidant exposure leads to an up-regulation of GSH synthesis. However, the vulnerability of neuronal cells to reactive nitrogen species is lost when neurones are co-cultured with astrocytes. Under such conditions, neuronal GSH status is enhanced and mitochondrial damage is minimised. Such enhancement of GSH availability appears to be facilitated by the release and trafficking of GSH and GSH precursors from astrocytes to neurones. Furthermore, this system appears to be responsive to oxidant stress, i.e. reactive nitrogen species exposure leads to an apparent increase in GSH trafficking. More recently, we have

demonstrated that exposure of astrocytes to glutamate also leads to an increase in GSH release form astroytes. In conclusion, it is postulated that GSH trafficking between astrocytes and neurones is sensitive to oxidative stress and that failure of this system will result in increased neuronal vulnerability leading to mitochondrial failure and ultimately cell death. Therapeutic intervention to maintain/enhance GSH trafficking may therefore be of benefit in neurodegenerative disorders.

S-26

Mitochondrial dynamics in neurodegeneration

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It is widely appreciated that mitochondria represent a special point of vulnerability in neurons, and that impaired mitochondrial function results in neuronal injury and death. One key mechanism for neuronal injury is the interruption of ATP generation, and a second may be the production of excess reactive oxygen species by mitochondria. However, there are many aspects of mitochondrial function within neurons that go beyond these important parameters, and these additional factors may contribute to neurodegenerative mechanisms. Mitochondria within neurons are very mobile, and we have observed that neurotoxins typically impair mitochondrial trafficking. Mitochondria can also undergo substantial changes in morphology, either as a result of disruption of the cytoskeleton or from the action of fission and fusion promoting proteins such as DRP1, mitofusin or OPA1. The location of mitochondria in neurons is subject to regulation, and the presence of mitochondria at synapses may be regulated by synaptic activity. Finally, it is also possible that the replication and degradation of mitochondria may be a target for the action of neurotoxins. In this presentation I will illustrate many of these phenomena. I will also discuss the challenges associated with establishing the cause and effect relationship between altered mitochondrial dynamics and neuronal death.

S-31

In vivo imaging of microglia reveals a prominent neuroprotective role in Alzheimer's disease

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Activated microglia is a prominent feature of Alzheimer's disease (AD) but their role in disease progression remains unknown. Here we studied the interactions between microglia, amyloid deposits and neurons by *in vivo* two photon and confocal microscopy in a mouse model of AD. We found that microglia play a critical role in phagocytosis of protofibrillar Aβ in newly formed deposits and around amyloid plaques. Also, by wrapping around plaques, microglia forms an insulating barrier that limits the ongoing binding of soluble Aβ and its aggregation into potentially more neurotoxic conformations. Deletion of CX3CR1, the chemokine fractalkine receptor which is selectively expressed in microglia, further enhances their phagocytic capacity and recruitment to Aβ deposits resulting in reduced Aβ deposition and attenuated synaptic loss. Together these results demonstrate that microglia activation in AD is a highly coordinated mechanism with a net protective effect whose modulation by CX3CR1 may represent an effective therapeutic target for AD.

S-32

Ischemic injury of oligodendrocytes in CNS white matter

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Injury to axon tracts in CNS white matter (WM) underlies much of the neurological disability seen with spinal cord trauma or ischemia. Ischemic WM injury involves two distinct pathophysiological mechanisms: an ionic pathway whereby axons are overloaded with Ca^{2+} via reverse Na^{+}/Ca^{2+} exchanger and Ca^{2+} channels, and an excitotoxic pathway that targets primarily oligodendrocytes and proceeds by overactivation of glutamate receptors. The mechanisms of excitoxicity in WM have been analyzed using the isolated mouse optic nerve (MON), a CNS WM area containing only myelinated axons. MONs from mature adult mice are studied in an interface chamber and superfused with artificial cerebrospinal fluid at 37°C. WM injury was assessed functionally by electrophysiology (i.e., stimulus evoked compound action potential (CAP)) and structurally using immunohistochemistry. Ischemia was achieved by exposure to oxygen/glucose deprivation (OGD). Axon function was severely reduced and structural abnormalities occurred after 60 min of OGD. Axons lost neurofilament immunofluorescence and oligodendrocytes died. Injury was largely prevented by blockade of AMPA and Kainate receptors (NBQX or CNQX). AMPA blockade alone (GYKI-52466 or Ca^{2+}-permeable AMPA receptor antagonist NAS) did not prevent injury, as is the case in other WM areas. Nor did blockade of kainate receptors alone (NS-102) offer protection in MONs. Blockade of NMDA type receptors did not protect against ischemic injury. Only when both AMPA and kainate receptors were blocked was ischemic injury and oligodendrocyte loss avoided, suggesting that overactivation of either AMPA or kainate receptors was sufficient to cause injury in this WM region. The mechanism of glutamate release appears to be reverse Na-glutamate exchange, probably from astrocytes. In mature adult WM, oligodendrocytes express AMPA, kainate and NMDA type glutamate receptors but only the former two mediate injury. These findings confirm that WM exhibits regional variability with regard to excitotoxic vulnerability and have therapeutic implications.

S-35

Reduced cerebral blood flow and oxygen consumption in cirrhosis patients with acute hepatic encephalopathy

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Hepatic encephalopathy (HE) is a common complication to liver cirrhosis that profoundly debilitates the patient's quality of life. Patients with HE are believed to have profound disturbances in brain hemodynamic and energy metabolism, but no studies have yet compared cerebral blood flow and oxygen consumption during HE in cirrhosis patients with HE. In order to investigate *in vivo* disturbances in brain energy metabolism during HE in patients with

cirrhosis, we combined measurements of cerebral blood flow (CBF), using ^{15}O-water PET, and measurements of brain oxygen consumption ($CMRO_2$), using ^{15}O-labeled oxygen PET, in the same individuals. The study comprised three groups of subjects: six patients with liver cirrhosis and clinically manifest HE, six patients with cirrhosis and no HE, and seven healthy subjects. PET data were co-registered with individual MR images for definition of specific brain regions. Mean arterial plasma ammonia was 129 ± 26 μM (mean ± SEM) and mean pCO_2 4.4 ± 0.3 kPa in the patients with HE, ammonia 69 ± 7 μM and pCO_2 4.8 ± 0.1 kPa in the patients without HE, and ammonia 26 ± 5 μM and pCO_2 5.2 ± 0.2 kPa in the healthy subjects, respectively. Whole-brain CBF was significantly reduced, to about two thirds, in the cirrhosis patients with HE in comparison to cirrhosis patients without HE and healthy subjects ($p < 0.01$), being 0.27 ± 0.01 mL blood/mL brain tissue/min in the patients with HE, 0.44 ± 0.02 mL blood/mL brain tissue/min in the patients without HE and 0.45 ± 0.02 mL blood/mL brain tissue/min in the healthy subjects. Analysis of regional distributions within and between the three groups of subjects showed that CBF in the cerebellum in the patients with HE was relatively less reduced than in the other regions. There were no significant differences between regional values of CBF within the two other groups of subjects. Whole-brain $CMRO_2$ was significantly reduced, to about two thirds, in the cirrhosis patients with HE in comparison to cirrhosis patients without HE and healthy subjects ($p < 0.01$), being 0.90 ± 0.07 μmol oxygen/mL brain tissue/min (mean ± SEM) in the cirrhosis patients with HE, 1.29 ± 0.09 μmol oxygen/mL brain tissue/min in cirrhosis patients without HE, and 1.29 ± 0.05 μmol oxygen/mL brain tissue/min in healthy subjects. There were no significant differences of whole-brain $CMRO_2$ between cirrhosis without HE and healthy subjects. There were no significant differences between regional values of $CMRO_2$ within any of the three groups of subjects. Individual CBF and $CMRO_2$ values were highly correlated, and both CBF and $CMRO_2$ were significantly negatively correlated to blood ammonia and pCO_2, respectively. The key result of the present study is that whole-brain blood flow (CBF) and oxygen consumption ($CMRO_2$) were both significantly lower in cirrhosis patients with HE than in cirrhosis patients without HE and healthy subjects. There were no significant differences of whole-brain CBF or $CMRO_2$ between the latter two groups. In conclusion, reduced whole-brain CBF or $CMRO_2$ were associated with the HE condition and not the cirrhosis as such.

S-39

Neuronal glial interactions in an animal model of childhood absence epilepsy

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The aim of this work was to investigate neuronal glial interaction in the Genetic Absence Epilepsy Rat from Strasbourg (GAERS) which is a model of childhood absence epilepsy. In GAERS all animals do not exhibit seizures until adult age and astrocytic and neuronal metabolism was studied before and after the onset of seizures. Furthermore, the ketogenic diet is known to reduce epileptic seizures and neuronal and glial interactions were investigated during the consumption of the diet. ^{13}C Nuclear magnetic resonance (NMR) spectroscopy was used to study neuronal glial interactions in the brain. Due to the differential distribution of enzymes and transporters in the brain it is possible to differentiate between neuronal and astrocytic metabolism after the simultaneous injection of [1-^{13}C] glucose and [1, 2-^{13}C] acetate and subsequent ^{13}C NMR spectroscopy analysis of the animal's brain extract. The level of glutamate was increased and that of GABA was decreased in the cortex of adult GAERS compared with non-epileptic controls. This change was not observed in young GAERS and is therefore probably linked to seizure activity. However, this alteration cannot solely be responsible for decreasing seizure threshold and enabling the generation of absence seizures since reducing the cortical glutamate content, as was observed in adult GAERS on the ketogenic diet (KD), did not suppress seizures. During consumption of the KD, the brain of GAERS adapts to ketone bodies as major source of fuel by reducing the glycolytic and oxidative metabolism of glucose as well as increasing metabolism in astrocytes. The exposure to the KD did not affect the cortical decrease in GABA content of adult GAERS fed a carbohydrate diet. However, the KD seemed to increase synaptosomal GABA content. This could mean that the generation of absence seizures in the thalamo-cortical loop is underlined by sufficiently disturbed glutamate and GABA homeostasis in the cortex in order to raise the brain's excitability. But neither disturbance of glutamate nor GABA homeostasis alone is responsible for generating absence seizures. Increased astrocytic metabolism was seen in adult but not in immature GAERS, suggesting that astrocytic alterations participate in the neuronal process leading to the occurrence of seizures.

S-40

Changes in the rates of the TCA cycle and glutamine synthesis in the brain with neurological disorder: studied by *in vivo* ^{13}C nuclear magnetic resonance spectroscopy

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It has been said that ^{13}C magnetic resonance spectroscopy (^{13}C-MRS) is a powerful tool for *in vivo* (noninvasive) measurements of rates of the tricarboxylic acid (TCA) cycle (Vtca) and glutamine synthesis (Vgln). However, only a little is known about the changes of Vtca and Vgln associated with neuronal disorders. We investigated the cerebral Vtca and Vgln using 2T MR scanner equipped with 2D ^{1}H-^{13}C HSQC sequence following the administration of [1-^{13}C] glucose in the monkey brain with hemiparkinsonism, in a patient with MELAS (the syndrome of mitochondrial myopathy, encephalopathy, lactic acidosis, and strokelike episodes), in the five intractable epilepsy patients, in four early Alzheimer's disease (AD) patients, and in eight young and five aged healthy volunteers. In the monkey studies, the average ratios of Vtca and Vgln on the left hemisphere (induced hemiparkinsonism by intracarotid infusion of MPTP) to those on the right hemisphere in post-MPTP-treated monkeys were 0.84 ± 0.09 and 1.37 ± 0.13, respectively. These results indicate that the loss of the dopaminergic innervation from the caudate putamen may modulate the overall glucose metabolism to glutamate and glutamine in the ipsilateral cerebrum. In the study of a patient with MELAS, we found the low glutamate production via the TCA cycle and high lactate synthesis through glycolysis. The studies of epilepsy patients revealed increased glutamine

synthesis compared with glutamate formation in a widespread cortical area with sustained epileptiform activities. The value of Vtca in the AD patients was 0.24 ± 0.06 μmol/g/min, and that in the young and aged healthy volunteers were 0.39 ± 0.19 and 0.37 ± 0.15 μmol/g/min, respectively. In conclusions, ^{13}C-MRS measurement following an administration of ^{13}C-labeled glucose can be applied for clinical use on diagnosis of mitochondrial dysfunction by monitoring cerebral glucose metabolism.

S-43

Tau phosphorylation antagonizes apoptosis by preserving survival proteins, a mechanism involved in Alzheimer's neurodegeneration

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Hyperphosphorylated tau is the major protein subunit of neurofibrillary tangles (NFT) in Alzheimer's disease (AD) and related tauopathies. However, it is not understood why the NFT-containing neurons seen in the AD brains do not preferentially die of apoptosis but rather degeneration even though they are constantly exposed to a proapoptotic environment, including β-amyloid (Aβ). The specific aim of the study was to explore whether tau phosphorylation affects cell survival and the underlying mechanisms. Cell culture and transfection, flow cytometry assay, MTT assay, LDH assay, cytochrome-c release assay, Caspase-3 activity assay, Hoechst staining of nuclear chromatin, Western blotting, Immunofluorescence staining, Sandwich ELISA, and site-specific mutagenesis were employed for the study. The cells overexpressing tau exhibit marked resistance to apoptosis induced by various apoptotic stimuli, including Aβ, H_2O_2, Staurosporine and Camptothecin. These reagents also caused correlated tau hyperphosphorylation and glycogen synthase kinase-3 (GSK-3) activation. GSK-3 overexpression did not potentiate apoptotic stimulus-induced cell apoptosis in the presence of high levels of tau. The resistance of neuronal cells bearing hyperphosphorylated tau to apoptosis was also evident by the inverse staining pattern of PHF-1-positive tau and activated caspase-3 or fragmented nuclei in cells and the brains of rats or tau-transgenic mice. Tau hyperphosphorylation was accompanied by decreases in β-catenin phosphorylation and increases in nuclear translocation of β-catenin. Reduced levels of β-catenin antagonized tau's anti-apoptotic effect, while overexpressing β-catenin conferred resistance to apoptosis. Expression of the hyperphosphorylated tau in N2a/APPswe attenuated prominently the Aβ-potentiated apoptosis, demonstrated by reduced nuclear fragmentation and inhibition of caspase-3/PARP, and elevated Bcl-2 and suppressed Bax and cytosolic release of cytochrome-c. Tau hyperphosphorylation leads the neurons to escape from an acute apoptotic death by preserving multiple survival proteins. Our study provides a model explaining why the tangle-bearing neurons in the AD brains do not preferentially die of acute apoptosis even though they are constantly exposed to an environment with enriched Aβ.

S-45

Therapeutic interventions for acute spinal cord injury in rats

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Traumatic spinal cord injury (SCI) initiates a complex series of cellular and molecular events that induce massive apoptotic cell death leading to permanent neurological deficits in human. Only one agent, methylprednisolone (MP), is currently being applied clinically to treat SCI. However, the clinical significance of recovery after MP treatment is unclear and must be considered in the light of potential adverse effects of its high-dose treatment. Therefore, other pharmacological treatments for acute SCI must be investigated. Our previous report indicated that minocycline reduces neuronal apoptosis and improves functional recovery through inhibition of proinflammatory cytokines after SCI. Recently we found that minocycline improves functional recovery after SCI in part by reducing apoptosis of oligodendrocytes via inhibition of proNGF production in microglia. Especially, p38MAPK was only activated in microglia, and minocycline treatment inhibited proNGF production by inhibition p38MAPK activation after SCI. Furthermore, minocycline treatment significantly inhibited $p75^{NTR}$ expression and $p75^{NTR}$-mediated apoptosis of oligodendrocytes, leading to inhibition of demyelination and axon degeneration. As other approaches for therapeutic interventions after acute SCI, the effects of PEP-1-SOD1 fusion protein and HP012, an extract of flavonoids from *Scutellaria Baicalensis Georgi* after injury was investigated. Systemic injection of the fusion protein significantly decreased the levels of ROS, carbonylation and nitrosylation of proteins after SCI. PEP-1-SOD1 treatment also significantly inhibited the mitochondrial cytochrome c release, and activation of caspase-9 and caspase-3 after injury. Furthermore, PEP-1-SOD1 significantly inhibited ROS-initiated apoptosis of ventral horn motor neurons and thereby improved functional recovery after SCI. These results suggest that PEP-1-SOD1 may provide a noble strategy for the therapeutic delivery of antioxidant enzymes that protects neurons from oxidative stress after SCI. Furthermore, oral administration of HP012 prevented apoptosis of neuron and oligodendrocyte by inhibiting the production of proinflammatory cytokines and ROS from microglia after SCI. Also, HP012 significantly attenuated the loss of axon and myelin and thereby improved functional recovery after SCI. Thus, our results demonstrated that minocycline, PEP-1-SOD1 and HP012 may provide us with therapeutic interventions for treating acute SCI.

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S-48

Ischemic tolerance in arctic ground squirrels: a hibernating species

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Arctic Ground Squirrels (*Spermophilus parryii*) tolerate cardiac arrest *in vivo*, and AGS hippocampal brain slices tolerate modeled

ischemia *in vitro*. To further elucidate the source of ischemic tolerance we minimized the influence of environmental factors by using hippocampal neurons differentiated from neural stem cells obtained from adult AGS. We report that these neurons are less susceptible to oxygen glucose deprivation and chemical hypoxia insult than other neural stem cell species. AGS stem cells were prepared from adult hippocampus (Nestin positive) and differentiated for up to 3 weeks until cultures contained at least 50% neurons (TUJ1 positive). For oxygen glucose deprivation, cultures were transferred to reduced glucose condition and were then sealed in hypoxic chambers, where O_2 was reduced to $< 0.6\%$. Normoxic cells were incubated in an unsealed chamber. After 48 h, cells were removed from their chambers and glucose added back to 5 mM and cells incubated at 37°C in air/5% CO_2 for another 24 h to model reperfusion. Cellular respiration, a gauge of cell number and viability, was quantified using Alamar blue fluorescence. Total cell and neuron numbers were obtained with an ArrayScan II (Cellomics, Inc., Pittsburg, PA, USA). Oxygen glucose deprived cells showed no sign of injury compared to control conditions. These same conditions were injurious to human neurons differentiated from neuronal stem cell precursors that showed significant loss of Alamar Blue fluorescence intensity and neuron numbers in normal glucose or low glucose media. AGS neurons treated with sodium cyanide and 3-nitropropionic acid (3NP) to inhibit aerobic metabolism showed no toxicity with minimal ATP loss. The AGS neuron tolerance observed under the above conditions could be accounted for by an ability to switch completely to anaerobic metabolism, a slowing of metabolic rate or a combination of all these two mechanisms. Remarkably, after modeled ischemia and reperfusion neuron numbers increased in AGS cultures suggesting that metabolic challenge stimulated neurogenesis. In conclusion, neurons differentiated from AGS stem cells tolerate ischemic-like insult suggesting that ischemia tolerance in AGS is due, in part, to inherent neuronal factors.

P1-01-01

Decreased turnover of glutamate, glutamine, and GABA precedes the occurrence of spontaneous seizures in an animal model of temporal lobe epilepsy

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Temporal lobe epilepsy (TLE) is the most frequent type of epilepsy in adults. The patient history often involves an initial precipitating injury early in life, followed by a latent phase, before spontaneous seizures occur. In the chronic phase of TLE, hypometabolism of glucose interictally and impaired glutamate homeostasis was observed, including glutamate–glutamine cycle dysfunction and increased release of glutamate during seizures. Using the kainate (KA) animal model of TLE, we also reported decreased glutamate turnover during the chronic phase. However, it is not known whether this glutamatergic hypometabolism is a consequence of seizures, or involved in the development of epilepsy (epileptogenesis). Thus, we set out to study intermediary metabolism in the KA model during the latent phase. KA treated rats were labelled and spectroscopy and HPLC on tissue extracts from hippocampal formation, entorhinal- and piriform cortices, and neocortex were performed. Decreased incorporation of ^{13}C label was observed in the corresponding metabolites. Whereas decreased turnover of glutamate and glutamine were seen in all brain areas studied. Decreased GABA turnover and increased content of branched chain amino acids were change specific for the mesial temporal lobe structures of KA animals. The mechanisms underlying the altered metabolic profile include impaired glycolysis, neuronal cell loss, mitochondrial dysfunction both in neurons and astrocytes, as well as impaired glutamine-GABA transfer. Glutamate-glutamine cycling appeared adequate and hypometabolism was more global and pronounced in the latent phase compared to previous findings in the chronic phase. In conclusion, the present study suggests that decreased turnover of glutamate, glutamine, and GABA is involved in epileptogenesis.

P1-01-02

Cerebrovascular endothelial dysfunction is a major source of neuronal cell death, oxidative/nitrosative stress and blood-brain barrier disruption in Wernicke's encephalopathy

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Wernicke's encephalopathy, a metabolic disorder characterized by selective neuronal cell death, glial activation and blood–brain barrier (BBB) breakdown, is caused by thiamine (vitamin B1) deficiency (TD) and leads to inhibition of the activity of the thiamine-dependent enzyme α-ketoglutarate dehydrogenase (α-KGDH) cerebral energy failure and lactic acidosis. Nitric oxide (NO) mediated oxidative/nitrosative stress is a common feature of many neurodegenerative disorders. Among the isoforms of nitric oxide synthase (NOS), the enzyme responsible for the synthesis of NO, the endothelial isoform (eNOS) was shown to be mostly implicated in the pathophysiology of TD. Knockout mice for eNOS (eNOS-/-) and wild-type (WT) mice were used to evaluate that eNOS derived NO is responsible for the oxidative/nitrosative stress, microglial activation and BBB breakdown observed in TD. Significant neuroprotection was observed in eNOS-/- mice in vulnerable areas, inferior colliculus and thalamic nuclei, compared to WT mice. Results revealed a significant attenuation of oxidative/nitrosative stress as shown by decreased protein nitration and nitrosylation in eNOS-/- mice. Expression of gp91-phox, a subunit of the NADPH oxidase complex involved in superoxide production, as well as CD74 (OX-6), an MHC class II antigen strongly expressed on activated microglia and infiltrating macrophages, where also decreased in eNOS-/- mice. A significant decrease of occludin protein expression was normalized in eNOS-/- mice, suggesting an attenuation of BBB breakdown. In conclusion, eNOS derived NO is a major contributing factor to the oxidative stress, microglial activation, BBB disruption and selective neuronal cell death occurring in thiamine deficiency. Also, that vascular endothelium dysfunction plays a key role.

P1-01-03

Mitochondrial abnormalities in lymphoblasts from autism

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Recent reports indicate the role of oxidative stress in autism and disturbance of energy metabolism has also been suggested. The free radicals, namely, reactive oxygen species (ROS) and reactive nitrogen species (RNS) are generated endogenously during oxidative metabolism and energy production by mitochondria in the body. While oxidative phosphorylation in the mitochondria generates superoxide anion, enzymatic oxidation of biogenic amines by monoamine oxidase in mitochondrial outer membrane produces H_2O_2. Damaged mitochondria not only produce more oxidants, but are also vulnerable to oxidative stress. Additionally, alterations in mitochondrial membrane potential (MMP) are involved in both apoptotic and necrotic cell death. We studied whether there is mitochondrial dysfunction in autism by analysing mitochondrial free radicals generation and membrane potential in the lymphoblasts. The lymphoblasts from autistic and control subjects were obtained from Autism Genetic Resources Exchange Program, and the cell lysates and mitochondria were prepared. MMP was monitored using the fluorescent dye rhodamine 123, a cell-permeable cationic dye, which preferentially partitions into mitochondria. ROS generation in the mitochondria was measured by the oxidation of dihydrorhodamine 123 to fluorescent rhodamine 123, while RNS were measured by using nitric oxide fluorometric assay kit. Elevated ROS and RNS levels were observed in the mitochondria of autistic lymphoblasts suggesting increased free radical generation. The mitochondrial membrane potential was also reduced in lymphoblasts from autism. This suggests increased mitochondrial oxidative stress and reduced MMP in autism. Such mitochondrial abnormalities may lead to defects in oxidative phosphorylation and energy metabolism in autism.

P1-01-04

Prevalence of antiphospholipid antibodies in ischemic stroke in young: a study from the Institute of Neurology, Sri Lanka

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Antiphospholipid antibodies (APLA) are associated with cerebral ischemia in young adults. Currently no previously published data is available in Sri Lanka with regard to this. The prevalence of APLA was studied in patients with first-ever ischemic stroke under 45 years of age. Lupus anticoagulant (LA) and anticardiolipin antibodies (aCL) were tested for to elude any indications of ALPA. Also, clinical, laboratory and radiological features were recorded. Demographic data (age, sex and race), clinico-investigative profile in routine and related specific investigations: hemogram, platelet count, activated partial thromboplastin time (aPTT), prothrombin time, chest X-ray, electrocardiogram, echocardiography, aCL, anti-nuclear antibody (ANA), serology for Venereal Diseases Research Laboratory (VDRL) and human immunodeficiency virus (HIV) were all recorded. aCL and LA tests were performed in 26 patients between the ages of 15–45 years and three patients below the age of 15 years. CL and LA was positive in a female, 34 years of age with left capsuloganglionic lacunar infarct having a history of systemic lupus erythematosous. LA was positive in one female patient, who developed left side hemiparesis at the age of 22 years 1 week after child birth. A computerised tomography scan showed a history of hypothyroidism on the right side cerebral infarct. Prevalence of 8% (2/26) of APLA positive was seen in the test group after their first ever ischemic stroke, indicating the importance of investigating APLA in stroke in the young.

P1-01-05

Persistence of decreased activity of mitochondrial complex I during long period following seizures induced in immature rats by homocysteic acid

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Recently, we have demonstrated mitochondrial complex I inhibition in cerebral cortex of immature rats during the acute phase of seizures induced by homocysteic acid (HCA) that persisted for at least 20 h after. It was of interest to determine whether the enzyme activity may recover during longer periods following the acute phase of seizures. Seizures [having a character of status epilepticus (SE)] were induced by bilateral icv infusion of DL-homocysteic acid (HCA, 600 nmol/side) in 12-day-old male Wistar rats with implanted cannulae. At desired times of survival after SE, mitochondria were isolated from the cerebral cortex of animals for determination of citrate synthase (CS) and respiratory chain complex activies. The results have revealed that a marked decrease (approximately 60%) of complex I (evident when expressed both as the specific activity and as a ratio to CS) persisted during the whole period studied. Activities of complex IV and CS remained unaffected. Inhibition of complex I activity was not associated with changes in complex I content. It can thus be assumed that modification of enzyme activity, most likely oxidative, is responsible for the observed inhibition. Administration of (S)-3, 4-dicarboxyphenylglycine (S)-3, an agonist for subtype 8 of group III metabotropic glutamate receptors, resulted in a partial, but significant attenuation of complex I inhibition. This treatment had also a partial antiepileptogenic effect. Thus, suggesting that the inhibited complex I might play a role in the process of epileptogenesis.

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P1-01-06

Early death of scrapie infected mice after induction with EAE

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During the years or decades of prion disease incubation, the at-risk individuals are certain to encounter diverse pathological insults, such as viral and bacterial infections or inflammatory processes. Whether prion disease incubation time, location and accumulation of PrP^{Sc} or otherwise the pathology of prion and other diseases are affected by the co-infection process is unknown. Experimental Autoimmune Encephalomyelitis (EAE) is used extensively as an animal model for Multiple Sclerosis (MS), as well as a model for brain inflammation. After induction it results in an inflammatory response comprising mononuclear cell infiltrates around venules, demyelination, gliosis and axonal loss. Damage to the brain and spinal cord is mediated by $CD4^+$ Th1 and inflammatory cytokines. In this work, EAE was induced in mice previously infected with prions (i.c. or i.p.) and observed daily for symptoms of EAE and scrapie or other neurological dysfunctions. As expected, MOG induced EAE in C57BL mice resulted in chronic paralysis of the tail and hind limbs. All control mice died after an incubation time of more than 200 days while co-infected animals died much earlier (90–181 days). Similar results were obtained for i.c. inoculation of prions. Brain histological examination of the co-infected mice showed both infiltrates, EAE and brain vacuoles, as seen in the scrapie controls. Western blot analysis did not show any detectable difference in the levels of PrP^C or PrP^{sc} in the brains of the co-infected animals. We conclude that co-infection of prions and inflammatory insults can result in early fatal neurological disease.

P1-01-07

Homocysteine-induced, glutamate receptor-mediated and transactivation-dependent extracellular-signal regulated kinase phosphorylation in cultured cerebellar granule neurons

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Hyperhomocysteinemia has been recognized as an important factor in the development of stroke and Alzheimer's disease. Under pathophysiological conditions homocysteine in plasma can rises to > 100 μM. Previously, it was reported that it at 100 μM causes ERK phosphorylation in hippocampal slices, but the signaling pathway was only partly determined. In the present work, we used primary

cultures of cerebellar granule cells, a preparation of glutamatergic neurons, to study the involvement of epidermal growth factor (EGR) receptor (EGFR) transactivation and glutamate receptors in homocystein-induced ERK phosphorylation. Transactivation is a process in which activation of Gi/o protein coupled receptors or an increase in free cytosolic calcium concentration ($[Ca^{2+}]_i$) leads to Zn^{2+}-dependent metalloproteinase-catalyzed shedding of an agonist at EGFR, which in turn stimulates EGF receptors on the same cell or its neighbor(s). We have found that the homocysteine at clinically relevant concentrations stimulated phosphorylation of $ERK_{1/2}$. This process was inhibited by MK-801, an NMDA antagonist, and by CNQX, an AMPA/kainate antagonist. The increases in $ERK_{1/2}$ phosphorylation were also inhibited by GM6001 (a metalloproteinase inhibitor, preventing 'shedding' of growth factors), by AG 1478 (a receptor tyrosine kinase inhibitor, preventing EGFR activation), suggesting transactivation of EGFRs. This study shows that the phosphorylation of ERK induced by homocysteine was an EGFR transactivation process, mediated by glutamate receptors, which may play a role in homocysteine-evoked acute neurodegeneration.

P1-01-08

The assay of urine 11-dehydro-thromboxane B2 in patients with recurrent cerebral infarction

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Urine 11-dehydro-thromboxane B2 (11-DH-TXB2) is the stable thromboxane metabolite reflecting the level of *in vivo* platelet activation. The purpose of this study was to investigate the change of urine 11-DH-TXB_2 level in patients with recurrent cerebral infarction and to compare the rate of aspirin resistance (AR) in recurrent and first-ever cerebral infarction patients. Eighty-four patients were divided into three groups: recurrent cerebral infarction group (group A; $n = 26$; age, 62 ~ 85 years), first-ever cerebral infarction group (group B; $n = 30$; age, 41 ~ 84 years) and non-stroke group (group C; $n = 28$; age, 42 ~ 84 years). Urine samples were collected before and after aspirin intake in groups A B, while only one sample was collected in group C. Urine 11-DH-TXB_2 levels were measured with the method of EIA. AR was defined as a 25% rise from the mean urine 11-DH-TXB_2 level of group C in infarction patients (group A and B) after taking aspirin for 7 days. The urine 11-DH-TXB_2 levels in group A and group B before taking aspirin were significantly higher than group C ($p < 0.05$). Moreover, the levels decreased significantly after the use of aspirin in groups A and B ($p < 0.01$). However, the difference of the rates of AR between group A and group B were not significant ($p > 0.05$). Thus, urine 11-DH-TXB_2 levels were increased in acute phase in both recurrent and first-ever cerebral infarction patients. Aspirin could reduce the urine 11-DH-TXB_2 levels significantly in acute infarction patients.

P1-01-09

Epileptiform activity exerts long-lasting effects on NMDAR and AMPAR subunit expression, distribution and interaction in neocortical cultures

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The purpose of our study is to explore systematic effects of early epileptiform activity on the formation and function of developing neocortical neurons at the cellular level. We use an *in vitro* model of early-life seizure, that is, single event of epileptiform activity induced by magnesium-free medium treatment in primary cultured rat neocortical neurons. First we extract total protein and detected the expression of NR1, NR2A, NR2B, GluR1, GluR2 and PSD-95, and then by using biochemical sub-cellular fractionation and immunoblot analysis, we further examined alterations of receptor composition and sub-cellular distribution. Co-immunoprecipitation and immunocytochemistry was applied respectively to detect changes in protein(s) interaction, neuronal morphology as well as excitatory synapse formation. When the total neuronal protein was examined, we found a decrease in expression of NR2B NMDAR subunits and PSD-95 ($p < 0.05$) shortly after insult (within 24 h). With the use of cell fractionation, we found that the cellular location of each NMDAR subunit (NR1, NR2A and NR2B), AMPAR subunit GluR1 and GluR2 and PSD-95 changed after the induced eplileptiform activity. Co-IP detection revealed a gradually enhanced interaction between PSD-95 and NR2A compared with NR2B from 7DIV to 21DIV. In addition, early-life seizure-like insults still exert effects on excitatory synapses number, size and distribution. Epileptiform activity may cumber normal development of neocortical neurons and preserve them at much more naïve period with less matured function and strength. These findings in an *in vitro* model of early-life seizure may inform rodent models of epilepsy, as well as the pathology of seizures in human neocortical development.

P1-01-10

Neuroaxonal dystrophy: clinico-pathological features of an inherited disorder of NZ Romney sheep

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The objective of our study is to describe the clinical and pathological features of a recently recognized axonal dystrophy of inbred Romney lambs in NZ. Lambs from the mating of four putative carrier rams to 25 closely related ewes selected from inbred lines of sheep that had produced affected lambs at low frequency for several previous seasons generated the study material. The lambs born from the experimental flock in 2006 were examined for neurological signs at 3–4 week intervals from birth to 6 months of age. The pathology (gross and microscopic) of the central and peripheral nervous tissues was assessed in 10 affected lambs. Selected lesions in two lambs were examined ultrastructurally. Affected lambs developed a slowly and irregularly progressive weakness and ataxia with abnormal proprioceptive responses that was recognisable soon after birth. The inability of affected sheep to maintain growth and body condition necessitated euthanasia between 4 and 10 months of age. No gross lesions were recognized in the nervous tissues although brain weight was reduced in comparison with unaffected control lambs. Spheroidal swellings of distal segments of axons were present throughout the spinal cord, brainstem and cerebellum, with greatest concentrations in gray matter associated especially with termini of long axons, particularly in cuneate, olivary and medial geniculate nuclear regions. A novel inherited neuraxonal dystrophy of sheep that is characterised by widely distributed spheroids, often densely concentrated, is described.

P1-01-11

Comparative proteome analysis of human cortex protein expression after severe traumatic brain injury

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To this date, specific molecular markers for early diagnosis and prognosis monitoring of craniocerebral injury in clinical medicine do not exist. The objective is to compare differential cerebral cortical protein expression of craniocerebral injury patients and normal subjects through the use of proteomics. Ten patients (six males and four females, 20–58 years old), with severe craniocerebral injury, were selected from June 2004 to December 2006. Surgery was performed 4–12 h after craniocerebral injury, and injured cortical tissues of the frontal and temporal lobes were resected for sampling. At the same time, control cortical tissues were collected from frontal and temporal lobes of two epileptic patients and two lateral ventricular tumor patients. Ten samples from injured patients and four control samples were compared through the use of proteomics. Total protein was separated by two-dimensional electrophoresis with immobilized pH gradients, and the differential protein expressions were compared using image analysis after blue-sliver staining. Differential protein spot expressions were analyzed with a matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI/TOF MS) and electrospray ionization-quadrupole time of flight mass spectrometry (ESI-Qq TOF MS). Two-dimensional electrophoresis of protein from cerebral cortex was performed and the image analysis system detected 21 differential protein spots. Differential protein spot expressions analyzed by mass spectrometry resulted in 17 differential protein spots that related to metabolic response, oxidative stress response, and signal transduction. It is shown that MALDI/TOF MS and ESI-Qq TOF MS are exceptional methods for evaluating differential protein expression. In conclusion the results indicated 17 different craniocerebral injury-associated proteins.

P1-01-12

Altered glutamatergic function in the chronic MK-801 model of schizophrenia

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Traditionally most animal models of schizophrenia have focused primarily on the phenomena linked to dopamine, but later studies have pointed towards a hypoactivity of NMDA (N-methyl-d-aspartate) receptors. Blockade of NMDA receptors by antagonists such as MK-801 and PCP causes behaviour that resembles some aspects of schizophrenia. Schizophrenic psychoses are therefore thought to be related to abnormal gluatmatergic function. In the present study rats were given daily doses of MK-801 (0.5 mg/kg) or saline over a period of 6 days. All MK-801 injected animals showed characteristic behavioural changes. Repeated injections did not have any effect on duration or severity of behavioural changes. Fifteen minutes before microwave fixation, rats were given MK-801 together with [1-^{13}C] glucose (543 g/kg). Analyses of extracts from the frontal cortex, hippocampus, thalamus, nucleus accumbens, amygdala and striatum were performed using ^{13}C and ^{1}H magnetic resonance spectroscopy (MRS), gas chromatography mass spectroscopy (GC-MS) and high performance liquid chromatography (HPLC). Clear alterations were detected in the glutamatergic function. Furthermore, aspartate and GABA metabolism were altered in frontal cortex.

P1-01-13

Plasma levels of tryptophan in patients with chronic fatigue syndrome

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Chronic fatigue syndrome (CFS) is a condition characterised by debilitating fatigue and other nonspecific symptoms resulting in significant disability, and its pathophysiology remains to be elusive. The development and persistence of CFS is related to a number of biopsychosocial factors that are likely to be heterogeneous in nature. The objective is to find out whether there is any change in the levels of tryptophan in CFS patients. Determination of the levels of tryptophan in the plasma of CFS patients was analyzed by HPLC. The patients chosen were included if they fulfilled the CDC criteria and the same critical criteria to the healthy people. The levels of tryptophan in CFS (32.03 ± 5.33) were increased than those in controls (22.49 ± 4.26) with a statistical significance ($p < 0.01$). This suggests that serotonergic dysfunction occuring initially from the metabolitic disturbance of trytophan in the plasma might be one of the pathogenetic factors.

P1-01-14

Anti-oxidative stress: role of electric mildly-warmed needle on fatigue-related rat model

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Oxidative stress is one of the main mechanisms causing fatigue. The electric mildly warmed needle (EMWN) therapy of inner Mongolian medicine is an effective way to treat fatigue-related diseases. The possible effect on the changes of free radicals in a rat model was investigated by using an exhausted swim test. Male SD rats were assigned to control, model and EMWN groups. Needles, electrically warmed, were applied to 'Dinghui' and 'Xinxue' points once every 2 days. On the 21st day, the exhausted swim time was measured. The day after the serum, liver and testitis were taken for detecting malondialdehyde (MDA), glutathione (GSH) and glutathione peroxidase activity (GSH-Px) and superoxide dismutase (SOD). The exhausted swim time in the model was reduced from control but then increased significantly for rats treated with EMWN group compared to the model. There was no significant difference in the MDA content for rats' liver and testitis between the three groups. However, it only increased significantly in the model's serum. As for GSH content and GSH-PX activity, the two indexes in serum, liver and testitis decreased for the model from control, while they all increased significantly in EMWN group compared to the model. The SOD activity was reduced in the model's testitis and again increased significantly in EMWN group. In conclusion, the EMWN therapy

could raise the exercise ability and relieve fatigue. It was also shown to improve the activity of antioxidant enzyme in liver, serum and testitis, which may suggest that the therapy could reduce the oxidative stress, and the corresponding damage, alleviating fatigue diseases.

P1-01-15

Changes of aquaporion-4 and aquaporion-9 expressions in the stage of the brain edema after severe burn

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The expression of aquaporin-4 (AQP4), aquaporin-9 (AQP9) protein and their mRNA in the stage of brain edema after severe burn was studied. Rats inflicted with 30% TBSA III degree scalding injury were taken as the model. The changes of Brain Water content (BWC) were detected by the dry-wet weight methods. The sections of burn brain were stained with H.E. to study the pathological changes. Immunohistochemistry (IHC) and *In Situ* Hybridization (ISH) methods were used to examine the changes of expressions of AQP4, AQP9 protein and their mRNA during different periods after severe burn. Correlations between BWC and AQP4, AQP9 expressions were made. H.E. staining showed that the brain tissue had swelling changes after severe burn. The space around neurons, glial and capillary became larger, the number of neurogliocyte increased, several cell nuclei were deeply dyed. BWC and the expressions of AQP4, AQP9 protein and their mRNA was increased 2-h post-burn and reached peak at 6 h after burn. The AQP4- and AQP9-positive cells were located in the choroid plexus, ependyma, hippocampus, supraoptic nucleus, paraventricular nucleus and other brain areas. After 48-h severe burn, the BWC, the expression of AQP4 and AQP9 were still higher than those in the normal condition. The expression of AQP4 and AQP9 were positively correlated with BWC respectively after severe burn. The results indicate that the increased expression of AQP4 and AQP9 has a relevant relationship with the formation of brain edema after severe burn in common.

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P1-01-16

ZnT3 mRNA expression and ^{1}H-MRS *in situ* studies of metabolic changes in weanling rats following heat stress and febrile convulsion

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Focal N-acetylaspartate (NAA), choline (Cho) and lactate (Lac) to creatine (Cr) detected by Proton Magnetic Resonance Spectroscopy (^{1}H-MRS) has been proven effective in the *in vivo* investigation of brain metabolites in temporal lobe epilepsy. The goal of this experiment was to test the hypothesis that in a heat stress animal model of convulsion, ^{1}H-MRS measurement of NAA and other metabolites, together with neuropathological findings, provides a sensitive *in vivo* method to monitor the progression of heat stress and the occurrence of febrile convulsion. Warm water induced acute heat stress model of seizure was developed and ^{1}H-MRS was used to measure the ratios of NAA, Cho and Lac to Cr. *In situ* hybridization was used to detect Zinc transporter (ZnT3) mRNA expression. The ratio of NAA/Cr and Cho/Cr were not significantly changed among hyperthermic (non-seizure) (HS), febrile convulsion (FC) and control groups. However, a significant increase in Lac/Cr ratios was observed in FC rats. ZnT3 mRNA expressions was detected in dentate gyrus of rat. As *in situ* increase of Lac is a putative marker of seizure-induced neuronal damage and ZnT3 is associated with mossy fiber sprouting in hippocampus, our results suggest that even a single febrile convulsion with short duration may remarkably influence neuronal metabolism and cause subtle brain injury, which is worth of further investigation.

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P1-01-17

14-3-3gamma is up-regulated by and protects neurons from *in vitro* ischemia

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14-3-3 proteins are abundant in brain tissues and the gamma isoform is mainly expressed in neurons. We have previously reported that 14-3-3gamma could be up-regulated in astrocytes by *in vitro* ischemia and attenuated ischemia-induced astrocytic death by binding to phosphorylated Bad. In this study, we studied the expression, function and underlying mechanism of 14-3-3 in ischemic neurons. The up-regulation of 14-3-3gamma in primary cultures of mouse cerebral cortical neurons exposed to oxygen-glucose deprivation (OGD) was demonstrated by real time RT-PCR, Western blot analysis and immunostaining methods. Other 14-3-3 isoforms (β, ε, η and ζ) were differentially regulated by OGD treatment. Blocking the interaction of 14-3-3 proteins with their ligands by over-expression diforpein exacerbated cell death in N2a neuroblastoma cells. Over-expression of 14-3-3gamma enhanced cell survival in OGD-treated N2a cells significantly while other 14-3-3 isoforms were less effective. Moreover, suppressing 14-3-3gamma expression by siRNA technique facilitated cell death in N2a cells upon OGD treatment. To study the underlying protective mechanism of 14-3-3gamma, we investigated the interaction of 14-3-3gamma with Bad, Bax, p53 and Ask-1. We found that 14-3-3gamma bound more Bax in neurons upon OGD incubation while the binding of 14-3-3gamma with Bad, p53 and Ask-1 did not altered evidently. Taken together, these data suggested that up-regulation of 14-3-3gamma is an important part of endogenous protective machinery in ischemic neurons.

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P1-01-18

Translocation of TRPC5 channels contributes to cholinergic-induced plateau potential in epilepsy

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Muscarinic stimulation generates prolonged depolarizations called plateau potentials (PP) in hippocampal pyramidal neurons, which is

an attractive candidate for a major intrinsic conductance observed during ictal phase of seizures. Transient receptor potential (TRP) channels form a superfamily of cation channels that display great diversity of activation mechanisms and selectivities. Despite extensive studies on these channels, little is known about their specific physiological roles. Muscarinic activation evokes TRPC5 to generate nonselective cation conductances and also enhances protein translocation and insertion in the plasma membrane. We hypothesize that the muscarinic induced membrane insertion and activation of TRPC5 channels are involved in the generation of plateau potentials in CA1 hippocampal pyramidal neurons. After the treatment of a muscarinic agonist, carbachol (CCH), the surface expression of TRPC5 channels was increased by more than 15 times. Atropine, the muscarinic antagonist, decreased this enhancement of TRPC5 surface expression. Whole cell patch clamping of CA1 pyramidal neurons were used in hippocampal slices to determine the contribution of TRPC5 currents to the tail current that generates the PP. The common TRPC5 antagonists 2-APB and SKF-96365 could significantly depress the tail current as well as PP. Interestingly, the PI3K inhibitor wortmannin, shown to block TRP channel translocation, could also decrease PP and surface expression of TRPC5 channels. The calmodulin inhibitor W-7 also blocked tail currents. In conclusion the rapid translocation of TRPC5 channels contributes to the generation of the cholinergic-induced PPs through a Ca^{2+}/calmodulin and PI3K-dependent pathway providing further understanding into the pathology of epilepsy.

P1-01-19

Correlation of angiogenesis with related factors expression following intracerebral hemorrhage

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Intracerebral hemorrhage (ICH) is a severe type of acute cerebrovascular disease. Further understanding the mechanisms of regeneration following ICH is beneficial to developing new therapy for the disease. This study investigates whether ICH can induce angiogenesis and the expression of angiogenesis-related factors (VEGF, Ang-1, TSP-1 and TSP-2) and their receptors (Flt-1, KDR/Flk-1, Tie-2, CD36). Animal models were injected with collagenase stereotaxically into right globus pallidus and an intracerebral hemorrhagic model was made. At certain times animals were randomly chosen to receive intraperitoneal injections of bromodeoxyuridine (BrdU), H&E stain. Immunohistochemistry was used to observe the temporal and spatial profile of angiogenesis, expression of angiogenesis-related factors and their receptors. New vessels appeared around the hematoma and extended into it from 7 days. BrdU-labeled nuclei in endothelial cells resided around the hematoma and the labeling peaked from 7 to 14 days. The expression of angiogenesis-related factors and their receptors were mostly located in endothelial cells around and in the hematoma. The mRNA of VEGF, Flt-1 and Flk-1 peaked at about 21 days, Ang-1 and Tie-2 continued rising to 28 days, TSP-1 peaked at 4 days, TSP-2 at 14 days, and CD36 at 4 and 21 days. These findings suggest that ICH can induce cerebral angiogenesis and that upregulation of angiogenesis-related factors and their receptors is involved in the dynamic modulation of new vessel formation.

P1-01-20

The signal transduction mediated by EPO and proinflammatory cytokines in the JAK/STAT pathway

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It is well established that erythropoietin (EPO) is a pleiotropic cytokine, which has a brain-derived neuroprotective effect in the central nervous system. Immune abnormality has a close relationship with cerebral palsy (CP), and may be even involved in the occurrence of CP. Our experiment demonstrated that the amount of EPO in CP children was lower than that in normal group, and the amount of proinflammatory cytokines in CP patients, such as IL-6, TNF-α, were higher. The signal transduction mediated by EPO that plays a neuroprotective role and mediated by proinflammatory cytokines that lead to brain damage share the common JAK/STAT pathway. Under acute stress, Massive proinflammatory cytokines forcibly occupy the JAK/STAT pathway, and simultaneously activate many negative feedback inhibition factors in the JAK/STAT pathway, such as SOCS protein, which have reciprocally chiasmal inhibition to EPO in the JAK/STAT pathway. Therefore, they inhibit or reduce the expression of EPO signal, and will eventually weaken the neuroprotective effect mediated by EPO. Our experiment suggests that, by promoting the secretion of brain-derived EPO and up-regulating the expression of EPOR in CP children (especially infants), the neurodevelopmental treatment (NDT) can increase the expression of intracellular signal molecules mediated by EPO, and promote nerve repair and brain function remodeling in the early intervented CP children.

P1-01-21

Pyruvate protects experimental stroke: the involvement of anti-inflammatory mechanisms via inhibition of NF-κB and MMP9

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Stroke is the third leading cause of death in the United States and existing therapy is limited. Pyruvate is a key intermediate in glucose metabolism, but is also a free radical scavenger. Prior work has shown that ethyl pyruvate is neuroprotective of middle cerebral artery occlusion (MCAO). The neuroprotective and anti-inflammatory effects of pyruvate following transient MCAO was assessed. Male Sprague–Dawley rats were subjected to 2 h of MCAO, and pyruvate was administered 10 min before reperfusion. Twenty-four hours later, infarct size, neurological deficits, physiological parameters, NF-κB translocation, microglial activation and neutrophil infiltration were evaluated. Pyruvate did not affect physiological parameters but significantly reduced infarct volume. This was accompanied by improvement in behavioral tests. Pyruvate treatment also reduced the number of neutrophils stained by MPO. Microglial activation was reduced by 40%. Isolectin B4 positive cells (to identify all microglia) were also reduced by 52%. Immunohistochemistry showed that pyruvate reduced numbers of

brain cells with nuclear NF-κB staining from 31% (MCAO) to 7% (MCAO+pyruvate). As a positive control rats were given LPS to cause brain inflammation without cell death. LPS increased nuclear staining of NF-κB and this was almost completely abolished by pyruvate. Pyruvate treatment significantly reduced NF-κB activation in LPS and MCAO groups. Western blots of MMP9, a NF-κB regulated gene, showed increased MMP9 protein by 4- and 3.70-fold for LPS and MCAO respectively from shams. Pyruvate treatment profoundly reduced MMP9 increases due to LPS administration and MCAO. These results suggest that neuroprotective effects of pyruvate on MCAO may be correlated to its anti-inflammatory effect, possibly at the transcriptional level.

P1-01-22

The effect of neurodevelopmental treatment on the recovery of neural function and transforming growth factor-β1 levels in children with cerebral palsy

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The present study is a probe into this mechanism by means of observing the effects of neurodevelopmental treatment (NDT) on the recovery of neurological functioning and transforming growth factor-β1 (TGF-β1) levels in children with cerebral palsy (CP) in attempt to contribute theoretically to the clinical NDT therapy of CP. Self-control method. Serum samples of 55 CP children, 33 neonates who suffered asphyxia and/or infection had accepted the NDT therapy for the first time for 3 ~ 4 months and 21 controls were obtained. Serum TGF-β1 levels were measured by the enzyme-linked immunosorbent assay double sandwich method (ABC-ELISA). Serum TGF-β1 levels of CP patients with NDT treatment were higher than those in the CP patients before they with NDT treatment and control group ($p < 0.01$, $p < 0.001$). Serum TGF-β1 levels of the CP children without NDT were significantly lower than those of controls ($p < 0.01$). Serum TGF-β1 Levels of neonates with CP risk factors were significantly higher than those of controls, those of the CP children without NDT and those of the CP children with NDT ($p < 0.01$). NDT may promote recovery of neural function and TGF-β1 synthesis in the brain of children with CP, indicating that CP as a traumatic factor had excited neurocytes around the focus to synthesis TGF-β1 and this synthesis was an instinctive protective reaction of CNS tissues toward the injury. On the other hand the interference of the NDT not only enhanced this protective reaction but also started to prepare materially for the subsequent functional reconstruction.

P1-01-23

The role of hypoxia in the differentiation of P19 embryonal carcinoma cells into dopaminergic neurons

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Nervous system development at early stage is in hypoxic environment. Very little is known about the role of hypoxia in neuronal development. P19 embryonal carcinoma (EC) cells are a well known model for early neuronal development. In this study we investigated the roles of hypoxia in dopaminergic neurons' differentiation derived from P19 EC cells. Results demonstrate that hypoxia increases the percentage of differentiated neurons, especially neurons of dopaminergic phenotype. To investigate the potential mechanism involved in hypoxia promoting dopaminergic neurons' differentiation, we detected the expression of hypoxia inducible factor 1α (HIF-1α), due to its key role in hypoxic response. The result shows that HIF-1α mRNA level increases after hypoxia exposure. It is known that HIF-1α regulates the expression of tyrosine hydroxylase (TH) gene through binding to its promoter. Therefore, we propose that the underlying mechanism of hypoxia promoting dopaminergic neurons' differentiation mediates by HIF-1α in response to hypoxia.

P1-01-24

Association study of schizophrenia with polymorphisms at four candidate genes

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Clinical studies have shown that there is a genetic contribution to the pathogenesis of schizophrenia. The molecular mechanisms of effective antipsychotic drugs and recent advances in neural development suggest that several dopamine receptor, serotonin receptor and opiate receptor genes might be involved in the disorder. In this study, we assessed the associations between schizophrenia and polymorphisms in the dopamine receptor 2 (DRD2), the serotonin 2A receptor (5HTR2A), the δ opioid receptor OPRD, and the μ opioid receptor (OPRM1) in 100 schizophrenic patients and 100 control individuals in an attempt to determine whether they have an association with the disease. Our results suggest that the polymorphisms at the DRD2, 5HTR2A, OPRD gene loci are unlikely to make our sample more genetically susceptible to schizophrenia. However, we found significant differences in microsatellite allele frequencies between schizophrenic and control groups for OPRM1 in the whole sample. The frequency of the Asn40 allele of OPRM was significantly increased in all schizophrenia patients (Fisher's Exact Test $p = 0.0000001418$) as well as in schizophrenia patients with no history of substance use (Fisher's Exact Test $p = 0.0014$). There were no associations of the Asn40Asp polymorphism of the μ opioid receptor with substance dependence among schizophrenia patients and normal control. This allelic association suggests that the functional Asn40 variant of OPRM may play a role in susceptibility to schizophrenia.

P1-01-25

The influence of neurodevelopmental treatment on erythropoietin levels in patients with cerebral palsy

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To investigate the serum and cerebrospinal fluid (CSF) erythropoietin (EPO) levels in the three different groups which include

cerebral palsy (CP) group [treated before and after neurodevelopmental treatment (NDT)], hypoxic ischemic encephalopathy (HIE) newborns group (CP high risk group), control group and the correlation between them. CSF and serum samples of 30 CP patients (before and after NDT) and 30 HIE newborns were collected. Meanwhile serum samples of 30 controls were obtained and kept at −70°C until the time of measurement. EPO levels were measured by the enzyme-linked immunosorbent assay double sandwich method (ABC-ELISA). The EPO levels in serum and CSF of CP patients with NDT treatment were higher than the CP patients before they treated with NDT treatment and control group ($p < 0.01$), the EPO levels in serum and CSF of HIE newborns group was higher than its control group ($p < 0.01$). There was no difference between CP group before they treated with NDT treatment and control group with regard to serum and CSF EPO levels ($p > 0.05$). EPO as a kind of neurotrophic and neuroprotective cytokine play an important role in the pathogenesis of cerebral palsy. Depending on the severity of the injury, the decrease for EPO production may have variable effects on neurons which may give rise to the current results. EPO can be as an independent biomarker that reflects curative effect of NDT. As the investigation into clinical applications for EPO progresses, a deeper appreciation for the novel roles that EPO plays in the brain should be acquired.

P1-01-26

Expression of the transcription factor Olig1 after focal cerebral ischemia in the rat: implications for demyelinated lesions repair

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Oligodendrocytes are myelin-forming glial cells and like neurons are highly sensitive to injury such as ischemia. Olig1 and Olig2 are related basic helix–loop–helix transcriptional factors that are expressed in the myelinating oligodendrocytes and their progenitor cells (OPCs) in the vertebrate central nervous system. The functions of Olig1 are required for maturation of OPCs to oligodendrocytes and the repair of demyelinated lesions while Olig2 participates in oligodendrocyte specification. The expression of Olig1 in the rat brain and the role of Olig1 during remyelination after middle cerebral artery occlusion (MCAO) was investigated. Using the immunohistochemistry technique, Olig1 proteins were shown to be widely presented in the cytoplasm of normal-appearing oligodendrocytes. However, when the rat brain underwent ischemia, the translocation of Olig1 into the nuclear at the edge of the lesions was detected. The expression of Olig1-positive cells on the ischemia brain started to decrease from after 6 h MCAO, which gradually declined until 7 days, and then increased throughout the remaining period of 21 days. Blue-silver staining was used and the degeneration and loss of myelin sheaths and axon was observed 6 h after cerebral ischemia. The integrated optical density ratio of the myelin sheaths staining in both sides showed that after 14 days focal ischemia, the demyelination reached the peak level. These results suggest that the Olig1 nuclear translocation in the early phase of focal cerebral ischemia and the increase of Olig1-positive cells in the late stage surrounding the lesion areas might be involved in demyelinated lesion repair and contribute to the functional recovery.

P1-01-27

Protective role of 14-3-3 proteins in transient global cerebral ischemia in rats

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14-3-3 protein family has seven mammalian isoforms: β, γ, ε, η, ζ, σ, τ and is highly expressed in the brain. 14-3-3 binds to more than 200 other proteins and is known to play critical regulatory roles in the survival and apoptosis of cells. 14-3-3 isoforms are now considered to have distinct functions in neurological diseases such as Alzheimer's disease, Parkinson's disease and Creutzfeldt-Jakob disease. We have previously reported that 14-3-3γ protected ischemic astrocytes from apoptosis. Here, we studied the protective role of 14-3-3 proteins in neuron in cerebral ischemic rats. Using immunohistochemistry, real-time RT-PCR and Western blot analysis, the involvement of 14-3-3 proteins, Bax, β-catenin and p53 in neuronal ischemic resistance was examined in rats subjected to 1 h transient global cerebral ischemia followed by 24 h reperfusion injury. Compared to controlateral counterpart, 14-3-3γ, η and β were selectively up-regulated while 14-3-3ζ and ε did not altered evidently in ipsilateral cerebral cortical neurons. The increase of Bax, β-catenin and p53 expression were also up-regulated in ischemic neurons. Moreover, the increase of 14-3-3 in ischemic neuron co-localized with the elevated p-β-catenin. Co-immunoprecipitation demonstrated that 14-3-3γ bound more p-β-catenin. Increased 14-3-3 level was found in survival neurons but not apoptotic ones. These data suggests that 14-3-3γover-expression acquires significant functions of neuronal protection in the injured brain, which provides a possibility to develop a novel therapeutic strategy for the patients with stroke.

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P1-01-28

Adjacent sections immunohistochemical study on the co-expression of Cholecystokinin and Prolactin in the brain of acute heat stress model of seizure in weanling rats

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To study the distribution of CCK-IR and PRL-IR cells in the brain following heat stress and febrile convulsion, warm water induced rat acute heat stress model of seizure was developed in this study. Rats were randomly divided into normal control group ($n = 8$) and hyperthermia-treated group ($n = 25$). The latter was further divided into febrile convulsion (FC, $n = 15$) and hyperthemic (non-seizure) groups (HS) ($n = 10$). Adjacent section immunohistochemical staining method was used to observe the co-expression of CCK and PRL. The results showed similar distributions and positive linear correlation of CCK-IR and PRL-IR in cerebral cortex of HS group ($Y = a + bX$; $a = 7.939$, $b = 1.36$, $r = 0.97$, $p < 0.01$). In addition, PRL-IR positive neurons were also found in midline thalamus of HS group, but no CCK-IR positive cells were seen there. While CCK-IR positive neurons increased significantly in the

whole cerebral cortex in FC rats than that in HS group ($p < 0.01$), more PRL-IR positive neurons were seen only in perirhinal and parietal cortex in FC group than that in HS group, without correlation between them. We conclude that a synergistic action of PRL and CCK may exist in the central control of heat stress progress. The distribution of PRL-IR in thalamus in HS group indicated that PRL might play more roles in the pathogenesis of heat stress than CCK does. The disappearance of the correlation between CCK-IR and PRL-IR in cerebral cortex of FC rats suggests the possibility that single febrile convulsion might cause subtle brain injury and is worthy of further investigation.

P1-01-29

Up regulation of Wnt signaling pathway in human gliomas

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Gliomas are the most common and deadliest form of primary brain tumors. Wnt/β-catenin/Tcf signaling pathway has been implicated in normal mammalian development and neoplastic transformation of colorectal and other malignancies but its role in glioma malignancy is poorly understood. We hypothesize that Wnt signaling pathway might play an important role in glioma tumorigenesis and its malignancy. Ten different human glioma biopsies were used to characterize the role of Wnt pathway components in glioma progression. We analyzed the levels of β-catenin, Lef1, Tcf4, cyclinD1, N-myc, c-myc, and c-jun, by western blotting and also checked the expression status of Dvl3 gene, an upstream activator of β–catenin, by semi-quantitative RT-PCR. In addition we performed IHC to analyze the status and localization of β-catenin, Lef1, Tcf4 and its targets in the human glioma specimens. Western Blot analysis revealed the upregulation of β-catenin, Lef1, Tcf4 and their target genes in the core tumor tissues and tumor cell lines in comparison to peripheral tissues. RT-PCR showed the elevated levels of Dvl3 m-RNA expression in tumor sample which is likely to be the key upstream activator of β-catenin. Immunohistochemical staining studies demonstrated that β-catenin was prominent in cytoplasm and nucleus, hallmark of Wnt pathway activation, while other proteins were localized in the nucleus of tumor cells. This reveals a significant correlation of β-catenin with other components in glioma progression. Therefore, indicate that Wnt/β-catenin/Tcf signaling pathway may be involved in glioma-genesis and correlates with its histological malignancy, which may serves as a target for glioma therapy.

P1-01-30

Genetic variant of BDNF (Val66Met) polymorphism attenuates neoangiogenic responses in cerebral ischemia

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Recent studies on the pathophysiology of ischemic stroke suggest the regulation of angiogenesis affects stroke outcome and recovery. In addition to its neuropoietic actions, brain derived neurotrophic factor (BDNF) promotes endothelial cell survival and induces neoangiogenesis in ischemic tissues. A single-nucleotide polymorphism (SNP) in the BDNF gene results in a substitution of methionine (Met) for valine (Val) at codon 66 (Val66Met). The mutation is common in human population and affects activity-dependent secretion of BDNF. The effect of genetic variant of BDNF (Val66Met) in ischemic outcome and neoangiogeneis in the brain was investigated. Male BDNF wild type (Wt: Val/Val) and mutant (Mt: Met/Met) mice were subjected to 30 min middle cerebral artery occlusion using an intraluminal thread method. Intra-ischemic cerebral blood flow was monitored by Laser-Doppler flowmetry before and during ischemia and reperfusion. Infarct volume (IV) and hemispheric swelling assessed 3 days after ischemia revealed no statistical difference between the strains (IV: Wt vs. Mt 36.9 ± 4.2, 27.7 ± 5.4 mm^3; % swelling: Wt vs. Mt 11.5 ± 2.7, 7.9 ± 3.5). However, ischemia-induced gene expression of CD36, which has an anti-angiogenic function, was significantly up-regulated in the mutant brain at 3 days ($p < 0.01$). In addition, the density of proliferating endothelial cells in the infract area was attenuated by 40% in the mutant mice. The results showed that BDNF is not involved in the development of acute ischemic lesion and activity-dependent BDNF secretion is associated with enhanced neoangiogenesis in the post-ischemic tissue. The study implies that the angiogenic mediator BDNF may be a therapeutic target for stroke recovery.

P1-02-01

Effect of hypothermia on astrocyte survival in a new model of post-traumatic neuroinflammation

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An *in vitro* post-traumatic neuroinflammation model was created by the coculture of leukocytes and primary cell culture of astrocytes, which had been traumatized by the scratch method. The scratch injury to astrocytes was produced by scratching a confluent culture with a 200 μL sterile plastic pipette tip, then gently washed to remove floating leukocytes. Quantitation of leukocytes adhesion was done by labeling leukocytes with Calcin-AM at 37°C for 30 min. The number of adherent cells was estimated from 10 fields along versus away from the edge of the injury using an inverted fluorescent microscope. Cell viability was estimated by LDH release and live/dead assay. Hypothermia (32 or 30°C) was started either immediately after trauma or 24 h after trauma (immediately after 30-min coculture with WBCs). We found that Leukocytes preferentially adhered to injured ($187.3 + 31.5$ cells/10 hpf) compared to uninjured astrocytes ($18.3 + 11.4$ cells/10 hpf). Optimal adhesion along scratch wound edge occurred 12- and 24-h post-injury, and expression of the adhesion molecule ICAM-1 was also increased at this time. Hypothermia at 32°C and 30°C had opposite effects on astrocyte survival following injury. Under hypothermia at 32°C, LDH release, dead astrocytes and leukocytes adhesion were reduced by 54%, 38% and 43% respectively. However, hypothermia at 30°C increased LDH release by 25% dead astrocytes by 33% and leukocyte adhesion by 150%. These results show that therapeutic hypothermia is useful in preventing secondary inflammation and cell death post-TBI and underscore the need for careful titration of

the level of hypothermia, as very low temperatures may exacerbate neuroinflammatory mechanisms.

P1-02-02

Functional neuro-energetic and brain imaging: how do astrocytes contribute to the signal?

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Understanding the central role of astrocytes in the distribution of energy substrates from the circulation to neurons is problematic because differentiating neuronal from astrocytic metabolites in a localized spectrum is difficult. Targeted dysprosium-grafted nanoparticles (Dy) were synthesized to be one cellular type specific. This allows us to distinguish neuronal metabolites from the astrocyte ones. Astrocytes and neurones were incubated with either glucose and [3-13C] lactate, or [1-13C] glucose and lactate for *in vitro* studies; that neurons preferentially consume lactate for their oxidative needs. At physiological concentrations, the relative contributions of glucose and exogenous lactate to neuronal oxidative metabolism were 25% and 75%, respectively. Rats were infused with [1-13C] glucose or glucose + [3-13C] lactate and HMQC spectra of brain extracts were recorded for *in vivo* studies. Surprisingly, there is a similar enrichment for Gln C2 and C3 when lactate was the infused substrate. For glucose, Gln C2 is always higher than C3, possibly due to the pyruvate carboxylase (PC) activity in astrocytes. Thus, lactate metabolism occurs in a brain compartment deprived of PC activity, most probably in neurons. Dy-DOTA and Dy-nanoparticles were tested to check the possibility to yield chemical shift modifications *in vitro*, both on metabolite solution and in cells, by 1H-NMR and HR-MAS spectroscopy and showed that the metabolites shifted by 0.2 ppm. The results obtained both *in vitro* and *in vivo* support the idea that astrocytic lactate could be a supplementary fuel for neurons under physiological conditions. The new approach using the physical properties of Dy realizes new insights on brain metabolic interactions.

P1-02-03

Branched chain amino acids restore hippocampal excitability after traumatic brain injury

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Traumatic brain injury (TBI) afflicts up to two million people annually in the United States, and is the primary cause of death and disability in young adults and children. Currently there is no treatment or therapy for the underlying causes of neurological dysfunction. By focusing specifically on the hippocampal formation, involved in both cognition and seizure susceptibility, this laboratory has partially elucidated underlying mechanisms which cause disruptions in the delicate balance of cellular metabolism and inhibitory and excitatory synpatic tranmission. Comparison of hippocampal slices from sham and TBI mice indicates a complex metabolic response following injury. Using a lateral fluid percussion injury model in mice, we examined regional changes in amino acid concentrations 7 days following injury. After TBI, in the ipsilateral hippocampus, the branched chain amino acids (Leucine, Isoleucine, and Valine) were reduced by 70.9%, 49.2% and 61.3%, respectively, while glutamate and glutamine levels were unchanged. BCAAs were exogenously applied to tissue slices and found to completely restore regional excitability as determined by extracellular recordings. Similar glutamine treatments had no effect suggesting a profound disruption of the neuronal-astrocytic glutamate:glutamine cycle after injury. Finally, administration of BCAAs to injured animals via the drinking water both restored hippocampal amino acid concentrations and, more importantly, completely reversed the altered regional excitabitliy shifts which are major components of the injury induced physiological footprint. The results implicate a multi-faceted response to TBI. The changes in BCAA levels, when coupled to the stable levels of glutamate, suggest a significant shift from their major role in neurotransmitter synthesis and recycling.

P1-02-04

Ischemia up-regulates 14-3-3γ in astrocyte through phosphorylation of c-Jun

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We previously reported that 14-3-3γ could be specifically induced by ischemia in astrocyte to protect them from ischemic injury. In this study, we attempted to elucidate the mechanism of this up-regulation of 14-3-3γ under ischemia. Using primary cultures of astrocyte, we found that Akt and p38 pathway were activated after 4 h of ischemia, while ERK and JNK were activated at 1–2 h which was consistent with the increase of 14-3-3γ mRNA under ischemia. We used inhibitors such as LY294002 for PI3K, U0126 for ERK, SB2035880 for p38 and SP600125 for JNK to clarify the pathway involved specifically with 14-3-3γ induction under ischemia. Result showed that only SP600125 could prevent 14-3-3γ up-regulation under ischemia. SP600125 inhibits JNK activation, thus prevent c-Jun phosphorylation; therefore, our findings demonstrated that c-Jun phosphorylation might play an important role in ischemia-induced up-regulation of 14-3-3γ.

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P1-02-05

Dexmedetomine-induced ERK phosphorylation occurs by EGFR transactivation in brain slices and in brain *in vivo*

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Dexmedetomidine is a potent and highly specific α2-adrenergic agonist, which in receptor binding experiments has a α2/α1

selectivity ratio of 1600 or several times higher than clonidine. The best known action of dexmedetomidine in brain is a presynaptic inhibition of noradrenaline release and cell firing in noradrenergic neurons, but only a minor fraction of α2-adrenergic receptors appears to be presynaptic. Our previous work suggested that dexmedetomidine-promoted ERK1/2 phosphorylation in primary cultures of mouse astrocytes is evoked by α2-adrenoceptor-mediated transactivation of epidermal growth factor receptor (EGFR), whereas it fails to activate ERK1/2 signaling pathway in primary cultures of neurons, probably indicating lack of expression of α2-adrenergic receptors (Li *et al.*, Brit. J. Pharm. **154:** 191–203, 2008). Here we report that this process occurs in brain slices, where ERK phosphorylation induced by dexmedetomidine is successfully inhibited by GM6001 and AG1478 (the potent Zn2+-dependent metalloproteinase and receptor tyrosine kinase inhibitors, respectively), suggesting a metalloproteinase-mediated shedding of membrane-bound EGFR-ligands. Also, that dexmedetomidine is capable to promote acute ERK1/2 phosphorylation in the mouse brain after intraperitoneal administration was confirmed by EGFR phosphorylation measured by immunoprecipitation. Our results raise the possibility that part of the reported beneficial effects by dexmedetomidine on neuronal survival during ischemia is due to an action on brain astrocytes that may trigger the release of glial neuroprotective substances, probably including heparin-binding epidermal growth factor (HB-EGF).

P1-02-06

Functional optical signals and neuro-astrocytic networks in the rodent olfactory glomeruli

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Changes in the neuro-glial activity induce variations in optical properties of brain tissues and imaging of Intrinsic Optical Signals (IOS) allows mapping of sensory-evoked activity. However, due to local blood-related changes, these signals are indirectly linked to neural activity. Odor-induced activation of olfactory glomeruli in the rodent olfactory bulb (OB) was mapped. We previously studied the cellular triggers odor-induced IOS and showed that glutamate release and uptake through astrocytic transporters are at their origin. An anatomo-functional properties of astrocytic networks based on gap jonctionnal (GJ) communication was studied. Immunohistochemistry and confocal microscopy indicated that the two major connexines (Cxs) forming GJ channels between astrocytes are differentially localized in the mouse glomerular layer. In acute slices, patch-clamp recordings of astrocytes and dye coupling experiments indicate that GJ communication is favored within glomeruli. Thus, astrocytic networks could contribute to define a glomerulus as a functional olfactory unit. Recording autofluorescence (AF) signals from the intracellular compartment relies on the AF properties of flavoproteins. An imaging set up was developed to record AF signals in the OB for the first time. First we carried out Monte Carlo simulations (MSC) to demonstrate the feasibility of AF recording. They allowed us to simulate the excitation and collection wavelengths of AF signals and the influence of the vascular responses on AF. In addition, we performed spectroscopic experiments to optimize these wavelengths. Unraveling the the neuro–astrocytic interactions and achieving cellular AF recordings in the olfactory glomeruli will allow further understanding of the link between cellular activity and functional signals in a defined network.

P1-02-07

Influence of integrin-blocking peptide on gadolinium- and hypertonic shrinking-induced neurotransmitter release in rat brain synaptosomes

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Integrins are a family of proteins involved in both neurodegenertation and neuroregeneration. These proteins are enriched in neuronal presynaptic endings but their functions to here are not very clear. It was shown that in neuromuscular junction integrins are able to mediate hypertonic shrinking induced calcium-independent exocytosis. We have investigated the contribution of integrins to calcium-independent neurotransmitter release induced by hypertonic solutions or polyvalent cations in isolated neuronal presynaptic endings which is model of central synapses. We confirmed that addition of 150–1000 mM sucrose to the incubation medium results in dose-dependent [^{3}H] D-aspartate and [^{3}H] GABA release. The polyvalent cations ruthenium red and gadolinium also lead to dose-dependent [^{3}H] D-aspartate release. Both hypertonicity and gadolinium evoked [^{3}H] D-aspartate release was calcium independent. We found that 200 μM of RGDS peptide, an inhibitor of integrins, decreased gadolinium induced [^{3}H] D-aspartate release by 26%. This compound had no effect upon hypertonicity induced release. The peptide RGES, a negative control for RGDS; genistein, an inhibitor of tyrosine kinases; and citrate, an inhibitor of lanthanides induced aggregation were ineffective in both cases. Moreover, RGDS did not influence gadolinium induced aggregation of synaptosomes. Hence, aggregation is excluded as a possible mechanism underlying the neurotransmitter efflux observed in our experiments. The results show that integrins did not influence hypertonicity-evoked [^{3}H] D-aspartate release, but partially mediated that evoked by gadolinium ions. This suggests that integrins can be receptor for heavy metals in presynaptic endings and probably can mediate neurodegeneration in heavy metals poisoning.

P1-02-08

Effects of homocysteine on metabolic pathways in cultured astrocytes

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Homocysteine is an amino acid that is an important risk factor for several neurodegenerative diseases such as Alzheimer's and Parkinson's disease. Increased homocysteine levels induce neuronal cell death in a variety of neuronal types. However, very few studies have probed the effects of homocysteine in astrocytes. The present study investigated the effects of homocysteine on primary cultures of astrocytes. Primary cultures of rat astrocytes were exposed to 400 μM homocysteine for 20 h. Metabolic extracts of cells were

prepared following a 4 h incubation in minimum medium with 5.5 mM [U-^{13}C] glucose in the presence or absence of homocysteine. The extracts were analysed using ^{13}C NMR. The expression level of pyruvate dehydrogenase kinase isoform 2 (PDK-2) and NAD(P)H levels were investigated following culture in the presence of homocysteine. Metabolomic analysis was performed using ^{1}H NMR spectroscopy and pattern recognition analysis. Following incubation with homocysteine there was a significant decrease (48%) in the ratio of flux through pyruvate carboxylase and pyruvate dehydrogenase (PDH) which was due to an increased flux through PDH. In addition, homocysteine culture resulted in a significant reduction in PDK-2 protein expression ($p = 0.01$). The increase in NAD(P)H fluorescence levels following stimulation by glucose were significantly higher in the homocysteine treated group ($p < 0.05$). Partial least squares discriminate analysis of the metabolic profiles showed that the most discriminating metabolites following homocystiene treatment were choline and hypotaurine. In summary, the results demonstrated that sub-lethal concentrations of homocysteine significantly enhanced flux through PDH and oxidative metabolism in astrocytes.

P1-02-09

Astrocytic 5-HT$_{2B}$ receptor stimulation by fluoxetine causes ERK phosphorylation mediated by EGF receptor transactivation

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A number of compounds that induce transactivation of the EGF receptor (EGFR) in astrocytes also have a neuroprotective effect. Recently, we found that fluoxetine causes EGFR transactivation. Although fluoxetine is known as a selective serotonin reuptake inhibitor (SSRI), which has low affinity for most 5-HT receptors including the 5-HT$_{2A}$ receptor, it has high affinity for the 5-HT$_{2C}$ receptor and for the recently demonstrated 5-HT$_{2B}$ receptor. The 5-HT$_{2B}$ receptor is the major 5HT$_2$ receptor present in primary cultures of astrocytes, and serotonin transporters are absent in these cells. We found that fluoxetine-induced ERK$_{1/2}$ phosphorylation in cultured mouse astrocytes was abolished by AG1478, an inhibitor of the epidermal growth factor receptor, and GM6001, an inhibitor of Zn-dependent metalloproteinase, suggesting receptor activity-mediated growth factor 'shedding' and transactivation of EGF receptors. ERK$_{1/2}$ phosphorylation was also inhibited by SB204741, an antagonist of the 5-HT$_{2B}$ receptor, with limited discriminating ability between 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors, but neither by the selective 5-HT$_{2C}$ receptor antagonist SB242084 nor by the selective 5-HT$_{2A}$ receptor antagonist M100907, ensuring that it specifically activated 5-HT$_{2B}$ receptors. The presence of calcium and PKC activity were required for stimulation. Furthermore, fluoxetine up-regulated mRNA and protein expression of c-fos and fosB. This effect was completely inhibited by AG1478, GM6001 and SB204741 as well as by U0126, an inhibitor of ERK phosphorylation, indicating that ERK$_{1/2}$ phosphorylation downstream of the EGF receptor is responsible for gene regulation. These effects may be involved in fluoxetine's antidepressant activity as well as its reported neuroprotective ability.

P1-02-10

Harmful increase of astrocytic Ca^{2+} trigger reactive oxygen species production

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Reactive oxygen species (ROS) are one of the key substances related to the initiation and progression of the Alzheimer disease (AD). Our hypothesis is that part of the ROS tissue content, in particular O_2- originates from protoplasmic astrocytes, and that its synthesis is triggered by intracellular Ca2+. By using two-photon laser microscopy, we measured spontaneous fluorescence oscillations produced by Ca2+ and O_2- indicators in hippocampal acute slices of APP and WT (control) mice. Astrocytes were loaded with Fluo-4 AM ester by using multicell bolus approach and O_2- indicators were locally delivery to the tissue by microinjection. We not only compared dynamic aspects of Ca2+ activity, but also found correlation between these two signals in both mouse types. Mice of several ages were used to explore variations in the intensity of Ca2+ and O_2- signals as a function of the amount of β-amyloid (Aβ) plaques. We provide evidences for a harmful increase of astrocytic Ca2+ correlated with the stage of the disease, previously examined by behavioral indexes. In order to verify if cation Aβ-triggered channels underlie such a harmful response, we applied locally zinc, a robust inhibitor if cation channels in many cell-types. Our results suggest an implication of astrocytic released-ROS in the progression of AD and open a new door for finding a plausible explanation of early vascular disorders reported in AD patients.

P1-02-11

Expression regulation of osmolarity changes on aquaporin-9 in astrocytes

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The expressions rules of aquaporin-9 (AQP9) and the molecular mechanism of its regulation in cultured rat astrocytes after exposure to different osmotic stress was investigated. The rat primary cultured astrocytes were divided into control, hypotonic and hypertonic groups and examined at interval times of 3 h, 6 h, 12 h and 24 h. Cells in the hypotonic group showed typical features of cell edema, and cell viability decreased, especially in lower osmolality group. The expression of AQP9 and its mRNA as well as the content of cAMP in the hypotonic cells were remarkably higher than the control ($p < 0.05$). The changes of AQP9 expression showed a positive relationship with AQP9 mRNA and with the content changes of cAMP. While, cells in the hypertonic group were shrinked, and cell viability decreased with a time-dependent manner. The expression of AQP9 and its mRNA was significantly higher than control before 12 h, but then decreased. At the same time point, the changes of AQP9 and its mRNA expression increased with a hypertonic level dependent manner, and the content of cAMP showed no changes. In conclusion, the expresson of

AQP9 had a direct correlation with osmotic changes. Hyponotic medium could induce cell edema, inhibit viability of astrocytes and up-regulate AQP9 and its mRNA, suggesting a mal-adaptation reaction. Hypertonic medium could induce cell shrinkage, inhibit viability of astrocyte, up-regulate AQP9 and its mRNA before 12 h, which may be important compensation in early stage hypertonic dehydration. These results imply that cAMP may participate in enhancing AQP9 expression in the hypotonic condition, but not in hypertonic condition.

P1-02-12

Fatty acid ligand in P2 protein – fact or fiction?

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P2 protein is a component of myelin which is located in the peripheral nervous system. Its distribution varies from species to species, between the peripheral and central nervous system and even from node to node. The tissue containing the richest source of P2 is myelin from intradural spinal roots of bovine. The 3D structure of bovine P2 was solved 20 years ago, at a resolution of 2.7 Å. However, some extra electron density was noticed, which was not accounted for by the amino acid chain. It was interpreted as an oleic fatty acid ligand. At that time, there was no biochemical evidence that this fatty acid could be a possible ligand. We worked with P2 protein purified from porcine (pig) spinal roots. The precipitant was 3.5 M ammonium sulfate (pH 6–8) and included in 'hanging drops' of crystallization trial. Resulted crystals were of a good diffraction quality, data was collected up to 1.8 Å. The initial model to solve the structure of porcine P2 was taken from bovine, of which amino acid sequence was fitted to the calculated electron density of the porcine P2 protein. Few substitutions between amino acids of bovine and porcine were noticed. The cDNA was further used to solve other ambiguities. Based on the electron density map, the potential substitutions were in positions T60 (P60), A84 (T84), V87 (T87), and K104 (N104). The extra electron density was detected and interpreted as a fatty acid ligand. The high-resolution atomic model indicated possible fatty acids: oleic or gamma-linolenic acid and their possible role in stabilizing myelin is discussed.

P1-02-13

Stimulation by vasopressin of ERK phosphorylation and vector-driven water flux in astrocytes is transactivation-dependent

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Regulation by vasopressin of water channels is a significant phenomenon in brain edema. Vasopressin acts on astrocytic G_q protein- and phospholipase C-coupled V_1 receptors. In mesangial cells, which also express the V_1 receptor, it stimulates cell growth by activating mitogen-activated protein kinase (MAP kinase) secondary to transactivation of the epidermal growth factor (EGF) receptor. Transactivation is an intracellular/extracellular process, in which activation of a G_q or a $G_{i/o}$ protein-coupled receptor leads to metalloproteinase-catalyzed shedding of an EGF receptor agonist, which stimulates EGF receptors on the same cell and/or its neighbor(s). Vasopressin signaling is mediated by transactivation also in astrocytes and whether such a transactivation is required for its ability to facilitate vector-driven water fluxes was investigated. Vasopressin concentrations between 10^{-12} and 10^{-6} M were found to lead to phosphorylation (activation) of extracellular regulated kinase 1 and 2 ($ERK_{1/2}$). Phosphorylation of $ERK_{1/2}$ could be completely inhibited by either AG1478, an inhibitor of the EGF receptor-activated tyrosine kinase, or GM6001, an inhibitor of Zn^{2+}-activated metalloproteinases, indicating the involvement of transactivation. Exposure to a hypotonic medium caused an immediate increase in cell water volume (demonstrated by decrease of fluorescence quenching of calcein), part of which was dependent upon the presence of vasopressin, added at a concentration of 1×10^{-8} M. This vasopressin-dependent component persisted throughout the duration of the experiment (22 min). The effect of vasopressin was abolished in the presence of AG1478, indicating its dependence upon transactivation, and by U0126 an inhibitor of the MAP kinase/ERK kinase (MEK), and thus of ERK1/2 phosphorylation.

P1-02-14

DREAM distribution in astrocyte, GABAergic neuron and glutamatergic neuron

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DREAM (Downstream Regulatory Element Antagonist Modulator) is involved in pain modulation. Its distribution patterns in different types of neural cells may implicate different roles of these neural cells in pain processing. We used primary cultures of cerebral cortical astrocyte (AS), cerebral cortical GABAergic interneuron (Inhibitory Neuron, IN) and cerebellar glutamatergic neuron (Excitatory Neuron, EN) to study the difference in distribution of DREAM. In IN transfected with DREAM-EGFP, laser scanning microscopy showed that DREAM was located in cytoplasm but not in nucleus. On the contrary, both EN and AS had strong DREAM expression in nucleus besides cytoplasm. Particularly in AS, DREAM signal in cytoplasm was lower than in nucleus. In IN and EN, DREAM was also located in neurites. Using DREAM antibody, these differences were confirmed in primary cultures of these three types of neural cells. The difference in DREAM distribution among AS, IN and EN indicated that these neural cells may play different roles in pain processing.

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P1-02-15

Astrocytes perform endocytosis to become hypertrophic

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Endocytosis refers to the cellular uptake of macromolecules and solutes into membrane-bound vesicles derived by the invagination and pinching off of pieces of the plasma membrane. One interesting function of endocytosis is its involvement in cell migration in developing tissues. It may be important for membrane rearrangements during migration, but evidences are not convincing. We noticed in scratch injured astrocytes that hypertrophic growth rate appeared to vary. Closer examination revealed that faster rates occurred in astrocytes exhibiting higher rates of endocytosis. Membrane ruffling was observed by time-lapse microscopy. Electron and atomic force microscopy together with dextran uptake confirmed that the endocytosis was macropinocytosis. Strong correlation existed between endocytosis and cell process migration. Inhibitors of phosphoinositide 3-kinase (LY294002), F-actin polymerization (cytochalasin D) and Raf-1 (GW5074) could reduce macropinocytosis. This suggested that endocytosis facilitated hypertrophy and cell processing migration. We also observed a higher rate of endocytosis in injured fibroblasts than astrocytes, its hypertrophic and migratory rates are faster than astrocyte.

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P1-02-16

Astrocyte poly (ADP-ribose) polymerase-1 activation causes glutamate excitotoxicity in neurons

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The nuclear enzyme poly (ADP-ribose) polymerase-1 (PARP-1) is activated in neurons and astrocytes in response to oxidative DNA damage, leading to cell death in cerebral ischemia. Reduction of PARP-1 activity could be a promising therapeutic target for stroke. Most of the mechanisms leading to neuron death in mixed cell population remain unclear. Since PARP-1 activation leads to nicotine adenine dinucleotide (NAD^+) and ATP depletion, we hypothesized that active glutamate uptake in astrocytes is compromised in conditions of elevated PARP-1 activity. We measured NAD^+ and ATP levels, and glutamate uptake capacity in cultured neonatal mouse astrocytes following PARP-1 activation by the DNA alkylating agent, 1-methyl-3-nitro-1-nitroguanosine (MNNG). Significant astrocyte death developed 6 h after MNNG exposure (100 μM for 30 min), so we limited our measurements to 4 h after MNNG treatment. The level of ATP decreased with time, diminishing by approximately 40% at 3 h and approximately 90% at 4 h after MNNG exposure. The depletion of NAD+ preceded declining cellular ATP content, as NAD+ levels were reduced by approximately 30% by an hour. In contrast, the depletion of ATP was not observed at this time point. Glutamate uptake capacity in astrocyte cultures was reduced in correlation with ATP depletion, declining by approximately 90% by 4 h after MNNG exposure. Bioenergetic depletion and reductions in glutamate uptake were not observed. Upon MNNG post-treatment, the survival was greatly improved for neurons that were plated on PARP-1-/- astrocyte cultures. Overall, PARP-1 activation in astrocytes contributes to bioenergetic depletion, elevated glutamate levels, and increased risk of glutamate excitotoxicity in neurons.

P1-02-17

Ammonia-induced activation of NADPH oxidase mediates cell swelling and glutamate uptake inhibition in cultured astrocytes

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Hepatic encephalopathy (HE) is a major neurological disorder that occurs in patients with severe liver disease. Ammonia has been strongly implicated as an important etiological factor in HE, and astrocytes appear to be the target of ammonia neurotoxicity. Astrocyte swelling and inhibition of glutamate transport are major findings in HE. While mechanisms mediating such defects are not well understood, reactive oxygen species (ROS) has been strongly implicated as an important factor. One potential source of ROS following ammonia treatment is the activation of NADPH oxidase (NOX). We therefore investigated whether ammonia activates NOX in cultured astrocytes, and whether such activation mediates astrocyte dysfunction. Cultures exposed to ammonia for different time periods significantly increased NOX activity in a biphasic manner. The initial increase in activity was observed at 5–30 min, followed by a decrease at 1 h, and returning to an increase at 4–24 h. Ammonia increased astrocyte swelling by 40.7% at 24 h and such swelling was significantly diminished by treatment of cultures with NOX inhibitors apocynin (APO) and diphenyliodonium (DPI). Similarly, cultures exposed to ammonia for 3 days showed an inhibition of glutamate uptake by 46.5%, and such inhibition was also significantly diminished by APO and DPI. Since ammonia is known to increase intracellular levels of Ca^{2+}, which activates NOX, we also examined the effect of the calcium chelator BAPTA-AM. This blocked the ammonia-induced NOX activity. These findings suggest that oxidative stress, mediated by NOX, contribute to the cell swelling and glutamate uptake inhibition associated with ammonia neurotoxicity.

P1-02-18

Astrocytic glutamate transporters and *in vitro* trauma

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The cellular and neurochemical mechanisms in traumatic brain injury (TBI) are heterogeneous and complex. Following TBI, astrocytic glutamate transporters are downregulated, resulting in increased extracellular glutamate concentration and the development of excitotoxic-mediated neuronal cell death. In order to study the relationship between GLAST and GLT-1 glutamate transporters when exposed to trauma, primary cultures of cortical astrocytes were subsequently exposed to a single, transient pressure pulse of compressed nitrogen gas using a Cell Injury Controller II Device, resulting in stretch-induced injury. Cells were studied in the presence of dibutyryl cyclic AMP which induces GLT-1. The resulting trauma resulted in a 45% increase in the uptake of [3H] D-aspartate, a non-metabolized analogue of glutamate 72 h following injury that was blocked by N-acetylcysteine treatment, suggesting an involvement of oxidative stress. Exposure of astrocytes to trauma also resulted in a time-dependent increase in the levels of GLT-1α and GLAST, suggesting the enhanced uptake was due to upregulation of these transporters. Treatment with minocycline was unable to prevent the increase in uptake, indicating that inflammation is unlikely to be responsible for this effect. However, increasing the magnitude of the injury from 1 to 4 atm resulted in an increase in GLT-1α at intermediate pressures but then decreased to normal levels at the highest pressure values. These results suggest that upregulation of glutamate transporters may be a normal protective response to trauma that is however limited by the force of injury, and possibly the presence of neurons, with increasing trauma leading to loss of this capability and susceptibility of the brain to excitotoxicity.

P1-02-19

Uptake of glutamate into astrocytic nucleus measured by RP-HPLC

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Excitatory amino acids glutamate (Glu) plays important roles in synaptic transmission in the central nervous system. Glu in astrocytes could be synthesized into glutamine, or utilized in TCA cycle for energy metabolism. To further explore the distribution of glutamate in astrocyte after being taken up, primary culture of cerebral cortical astrocytes were treated with 1 mM Glu, the Glu, glutamine (Gln) and taurine (Tau) contents in both intact astrocyte and isolated nuclei were measured through isocratic reversed high performance liquid chromatography (RP-HPLC) with fluorimetric detection. Amino acids were derivatized with phthaldialdehyde (OPA). Glu, Gln and Tau derivatives were separated with a Phenomenex LUNA ODS 2 column (250 × 4.6 cm, 5 μm). Our findings showed that Glu content increased in intact astrocyte after Glu treatment, and Glu content in the nucleus isolate was significantly increased from 0.5 to 2 h. There were no changes in Gln and Tau content in both intact astrocyte and nucleus isolate. Our results demonstrated that astrocyte could efficiently take up exogenous Glu which in addition to being metabolized in the cytoplasm, could also get into nucleus of the astrocyte.

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P1-02-20

Astrocytic nuclear swelling in respond to extracellular glutamate

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Excitatory neurotransmitter glutamate could induce astrocytic swelling *in vitro* and *in vivo*. In present study we found glutamate could trigger nuclear swelling in astrocyte. This effect was glutamate specific. The swelling rate was exogenous glutamate concentration dependent. It was mediated by glutamate transporters, but not ionotropic/metabotropic glutamate receptors on the plasma membrane. We confirmed glutamate-induced nuclear swelling with isolated astrocytic nuclei, indicating nuclear swelling was independent from cytosolic swelling. Immunostaining and HPLC measurement showed glutamate content in astrocytic nucleus elevated during swelling, suggesting glutamate entered nucleus to induce this effect. Interestingly, we found that glutamate worked together with aquaporin-1 to induce nuclear swelling. Aquaporin-1 was found expressed in astrocytes and concentrated in nuclear envelope. Therefore, present study revealed an unknown glutamate signaling mechanism in astrocyte, through which astrocytic nuclear functions were directly regulated by exogenous glutamate.

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P1-02-21

Expression of functional hyperpolarization-activated channels (HCN channels) in mouse cortical astrocytes

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Hyperpolarization-activated cyclic nucleotide-gated (HCN) channel are integral membrane proteins that assemble as tetramers to form channels in cardiac conduction tissue and nerve cells. It mediates hyperpolarization-activated cation current, Ih, which is shown to play an important role in regulating intrinsic neuronal excitability in the brain. In this study, we identified functional HCN channels in

astrocytes and examined their possible roles in these non-excitable cells. Although all four HCN subtypes HCN1-4 were detected in mouse cortical astrocytes culture by Real-time PCR, the dominant expression of HCN isoforms in astrocytes were HCN2 and HCN4, Electrophysiological study showed that astrocytes expressed a hyperpolarization-activated inward current which could be blocked by external Cs+ and ZD7288, two Ih blockers. Single-cell expression analysis combined with the whole cell patch-clamp revealed heterogeneity of astrocytes in the HCN subtypes expression. More than 80% astrocytes co-expressed HCN2 and HCN4 isoforms mRNA, suggesting heteromeric HCN2/4 channels existed in most astrocytes. 21.74% astrocytes expressed HCN1 isoform demonstrating relative depolarized resting membrane potential (RMP) and active whole cell current pattern, whereas other HCN1 negative astrocytes showed hyperpolarized RMP and relative passive electrophysiology. There was an obvious downregulation of HCN1, 2, 4 expressions in astrocytes during development, accompanying by astrocytic RMP hyperpolarization during the same period. Our study demonstrated that HCN channels might play an important role in modulating astrocytes RMP, thus might take part in controlling a series of physiological function including neurotransmitter release. The different HCN expression may contribute to the long-known heterogeneity of astrocytes.

P1-02-22

NDAP appears to cause apoptosis and localize in endoplsamic reticulum

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NDAP (neuronal development-associated protein) is a protein first identified in mouse cerebral cortical neurons in our laboratory. We previously reported that cells overexpressing NDAP died of apoptosis. For further understanding of the function of NDAP, we transfected astrocytes in primary culture and followed the subcellular localization of NDAP-EGFP. We found that NDAP-EGFP was localized on the endoplasmic reticulum (ER). Various NDAP-EGFP deletion truncates were constructed according to sequence analysis (NDAP $\Delta_{1–21}$, NDAP $\Delta_{1–21}/\Delta_{528–570}$, NDAP $\Delta_{1–527}$, NDAP $\Delta_{1–21}/\Delta_{520–570}$, NDAP $\Delta_{1–519}$, NDAP $\Delta_{1–21}/\Delta_{270–570}$ and NDAP $\Delta_{1–269}/\Delta_{520–570}$). All the constructs, except NDAP1–21/270–570, exhibited a similar ER distribution as the full length NDAP. NDAP1–21/270–570 was also detected on the cell membrane, indicating the loss of ER retention ability. Through overexpression of NDAP-EGFP and the deletion truncates experiments, we found that high levels of NDAP-EGFP or truncates could lead to apoptosis in astrocytes, COS7 and HEK293. Truncates NDAP $\Delta_{1–527}$ and NDAP $\Delta_{1–519}$ (mainly including the transmembrane region and C-terminal, respectively) displayed less degree of apoptosis than other truncates. Our study suggested that NDAP localized in ER and its N-terminal sequence contributed to its apoptotic function.

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P1-02-23

Expression of aquaporin 4 after scratch-wound in cultured rat astrocytes

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Aquaporin 4 (AQP4) is a predominant water channel protein in mammalian brain, localized in the astrocyte (AST) plasma membrane. In this study, we investigated the change of AQP4 expression level following the scratch-wound in cultured rat AST. The secondary cultured AST prepared from newborn Wistar rat cerebral cortex were scratched with plastic pipette tips. The morphologic change of AST was observed through microscope at 1 h before and 1, 12, and 24 h after injury, meanwhile the lactate dehydrogenase (LDH) leakages in the cultured medium were determined using a LDH assay kit, the change in the expression of AQP4 protein and mRNA were measured by western blot and real-time quantitative polymerase chain reaction. Immediately after injury the edge of the scratch was lined with irregularly shaped cell. Twelve hours after injury the AST processes extended to cell-free area, and elongated further at 24 h after injury, with presented of new generated cells in the denuded area. At different times after injury, the LDH leakages of the experiment groups were higher than that before injury ($p < 0.05$), and were higher than that of the control group ($p < 0.05$). The AQP4 protein expression is lowest at 12 h after injury and subsequence the expression is slowly increased. The AQP4 mRNA expression remained unchanged at 1 h after injury, but a marked decreased at 12 and 24 h after injury ($p < 0.05$). In conclusion, the expression of AQP4 is significantly decreased after scratch-wound in cultured rat astrocytes.

P1-02-24

A functioning glycolysis is important for maintenance of glutamate transport in cultured astrocytes

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Glutamate released from neurons into the synaptic cleft is predominantly taken up by surrounding astrocytes. This transport is driven by the Na+ gradient and is thus energy requiring via the Na+/K+ ATPase. Energy generating pathways that may be involved in providing ATP for maintenance of the Na+ gradient and subsequent transport of the glutamate analog D-aspartate (D-asp) in cerebellar astrocytes were examined. The transport process was monitored as homo-exchange of preloaded D-[3H] asp using a superfusion paradigm in which the cultures were exposed to pulses (6×10 s) of D-asp (1 mM). The homo-exchange process could be blocked by treo-benzyloxyaspartate (TBOA, 1 mM), a glutamate transport inhibitor. Iodoacetate (IA, 1 mM), fluoroacetate (3 mM) and 1, 4-dideoxy-1, 4-imino-arabinitol (DAB, 400 μM) were used as tools to inhibit glycolysis, TCA cycle and glycogenolysis, respectively. Also, the effects of different energy substrates were tested. The capacity for transport of D-[3H] asp was not affected by inhibiting

glycogenolysis and TCA cycle and surprisingly, neither did 1 h of aglycemia. The addition of IA significantly compromised the capacity for transporting D-asp both in the presence and absence of glucose, indicating an important role of glycolysis for the transport of D-asp. Observations suggest that a functioning glycolysis is important for optimal glutamate transport in astrocytes. Western blot of synapto-gliosomes showed that the glycolytic enzymes glyceraldehyde-3-phosphate dehydrogenase and phosphoglycerate kinase are present in the plasma membrane strengthening the hypothesis that ATP from glycolysis is important for the function of the Na+/K+ ATPase linked to the transport of glutamate in astrocytes.

P1-03-01

Modelling of astrocytic metabolism in primary cultures subjected to ischemia: merging ^{13}C NMR spectroscopy and metabolic flux analysis

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Cerebral ischemic stroke is a major cause of death in industrialized countries. The aim of this work was to identify and quantify intracellular metabolic fluxes in astrocyte cultures subjected to an ischemic insult. For that, a metabolic model recently developed by our group to study astrocytic metabolism was used. This approach applies metabolic flux analysis, together with ^{13}C-NMR spectroscopy and extensive measurements of extracellular metabolites consumption/secretion rates, to estimate metabolic fluxes. Cultured rat astrocytes were subjected to 5 h ischemia by removing oxygen and assuring the absence of glucose. To mimic the reperfusion period, culture medium was replaced by fresh medium containing [1-^{13}C] glucose. Cell supernatant samples were harvested at several time points for later quantification of total glucose, lactate and amino acids. ^{13}C NMR spectroscopy analysis was also performed to determine the PC/PDH ratio along the culture. It was possible to infer intracellular flux distributions both before and after the ischemic insult and, for example, to disclose the impact of variations of the PC/PDH ratio on these fluxes along time. Modelling identified two distinct metabolic phases after ischemia. In the first phase, a metabolic shift towards a more oxidative use of glucose was observed, while in the second, a decrease in the TCA cycle rates demonstrated that cells were able to partially overcome the insult, as they evolved into a state closer to initial physiological conditions. This work presents a consistent and simple approach providing a reliable characterization of the intracellular astrocytic metabolism under a stress situation.

P1-03-02

Ascorbic acid inhibits glucose transport in neurons but not in astrocytes: possible participation of glucose transporter 3

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Ascorbic acid (AA) is highly concentrated in the CNS. Previously, we demonstrated that intracellular AA is able to modulate neuronal glucose utilization between resting and activity periods and intracellular AA inhibits glucose uptake in neurons during glutamatergic synaptic activity was observed. Glutamatergic activity induces ascorbic acid depletion from astrocytes and other cerebral reservoirs. The AA effect in neurons and astrocytes is explored. We analyzed the expression of glucose transporters (GLUTs) and ascorbate transporters (SVCTs) in primary cultures of cortical neurons and cortical astrocytes. Neurons expressed GLUT1 and GLUT3, while astrocytes only expressed GLUT1. Both cell types expressed SVCT2. Functional analysis, using radioisotope-based methods, confirmed the presence of glucose and ascorbate transporters. We also analyzed desoxiglucose (DOG) uptake in cells preloaded with AA (intracellular AA) or co-incubated with AA (extracellular AA) and glucose analogue. Only intracellular AA was able to inhibit DOG transport in neurons. However, in astrocytes, AA did not inhibit DOG uptake under any of the conditions mentioned. Finally, we analyzed DOG transport in astrocytes expressing GLUT3 by transient transfection. We observed GLUT3-EGFP expression in plasma membrane in cultured astrocytes by confocal microscopy. Moreover, radioisotope-based methods confirm that GLUT3-EGFP was functional in these cells. During experiments in presence of intra or extracellular AA, we observed that AA inhibited DOG transport in GLUT3 expressing astrocytes. In conclusion, we have determined that neuronal glucose transport is inhibited by AA accumulated inside the cells expressing GLUT3. Therefore, we propose a model where AA is able to modulate glucose uptake by inhibition of GLUT3 function.

P1-03-03

Measurement of regional brain metabolism *in vivo* in the hyperglutamatergic transgenic mouse brain overexpressing glutamate dehydrogenase 1 using high resolution ^{1}H MRS

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Aging is associated with increased mitochondrial generation of reactive oxygen species (ROS), oxidation of mitochondrial DNA (mtDNA) and mitochondria-associated cellular oxidative stress. Excessive extracellular accumulation of glutamate (Glu) leads to neuronal oxidative stress and excess ROS formation. Both baseline and depolarization-induced release of Glu is higher in aged brain than young brains, which link aging and Glu signaling in the CNS. To determine how excess Glu may affect neuronal function and survival in the aging brain, we utilize hyperglutamatergic transgenic (Tg) mice that have extra copies of the mouse gene for Glu dehydrogenase 1 (Glud1), a mitochondrial enzyme that is a possible rate-limiting step in the biosynthesis of Glu. Neurochemical profiles were measured in the hippocampus and striatum using high resolution 1H MRS at ages of 62–64 weeks, 74–76 weeks and 82 weeks. Concentrations of NAA ($p = 0.03$) and creatine (Cr) + phosphocreatine (PCr) ($p < 0.001$) were significantly lower in the hippocampus of Tg than wild type (wt) mice at the age of 62–64 weeks. The lower concentration of Cr + PCr appeared to be mainly due to PCr being lower rather than Cr. In aging, differential trends in neurochemical concentration changes between Tg and wt mice were observed. These changes in Glu and GSH in Tg brain were in excess of those observed in wt mice. In conclusion, these selective regional changes of neurochemicals in the aging mouse

brain with a hypergulatamatergic state are in agreement with the notion that mitochondrial damage and ROS generation occurs due to Glu hyperactivity in selective brain regions.

P1-03-04

Transcriptional activation of the galactosyltransferase B3galt2 gene in rat cortical neurons requires activation of ERK and MSK1 kinases and phosphorylation of histone H3

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Glycosylation plays a pivotal role in development, differentiation, and malignancy. Glycosyltransferases are enzymes that catalyze transfer of carbohydrate groups from donors to acceptors. Expression of a galactosyltransferase gene, UDP-Gal:β GlcNAc β-1, 3-galactosyltransferase (B3galt2), is developmentally regulated in the mammalian brain and deletion of the gene leads to behavioral abnormalities. Our previous work has shown that the transcription factor CREB and both CaMK(s) and ERK1/2 signaling pathways are involved in an enhanced B3galt2 gene expression in rat cortical neurons induced by plasma membrane depolarization. In this study, the pathways linking plasma membrane depolarization to transcriptional activation of the B3galt2 gene were further investigated. Q-PCR, Western blot analysis, and immunohistochemistry were used to examine B3galt2 gene expression and phosphorylation of ERK, MSK1 and Histone H3 in rat cerebral cortical cultures and the mouse hippocampus. Correlations among a rapid, ERK-dependent MSK1 activation in nuclei, phosphorylation of serine 10 of the histone H3, and B3galt2 gene induction were identified. In conclusion we found the ERK-dependent chromatin remodeling mechanism is likely to be involved in the transcriptional activation of B3galt2 gene in the brain.

P1-03-05

Prevention of hypoglycemia-induced lipoperoxidation by ketone bodies

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Ketone bodies play a key role in mammalian energy metabolism as intermediates in the synthesis and breakdown of fatty acids. Normally ketone bodies blood concentration during adulthood is very low, although it can rise as a result of starvation, ketogenic diet or a ketotic infusion. Whenever ketone bodies levels increase, the adult brain is capable of using them as alternative metabolic substrates. For this reason their neuroprotective potentiality has been proved in diverse neurotoxic conditions. Recently, the ketogenic diet efficiently reduced hypoglycemia-induced neuronal death in young rats. The mechanisms underlying the protective effect of these compounds are not completely understood, although their metabolic contribution has been emphasized. Hypoglycemia is normally a transitory condition but it can turn chronic in patients with hormonal alterations, or in type 1 diabetic individuals exceeding their insulin dose. Selective brain damage occurs during hypoglycemia only after an isoelectric coma of at least 20 min. During this period energy failure, ionic imbalance and excitatory amino acid release take place. Animal studies using the insulin-induced hypoglycemia model have suggested the presence of oxidative stress even before the onset of coma. Based on this observation we evaluated the presence of lipoperoxidation in diferent brain regions of rats subjected to insulin-induced hypoglycemia, and tested the potentiality of ketone bodies to prevent it. Results show increased lipoperoxidation levels are only in brain regions vulnerable to hypoglycemia and a diferential protective capacity of ketone bodies, showing both isomers of β-hydroxybutyrate better protection than acetoacetate. This suggests an antioxidant action of ketone bodies.

P1-03-06

Measurements of equilibration between oxaloacetate and fumarate in awake rat brains

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Studies of brain metabolism have been facilitated by the use of carbon 13 labeled precursors such as glucose and acetate to monitor flux through metabolic pathways. The precursor can be infused into an anesthetized animal and carbon 13 spectra of the brain followed as a function of time in order to estimate flux through the citric acid cycle, glutamate/glutamine cycle, and CO_2 fixation. Previously, these types of studies have entailed complex modeling and assumptions about the stoichiometry of anaplerotic incorporation of carbon 13 from CO_2 into glutamate. Also they have been limited by the difficulty involved in monitoring the carbon 13 enrichment in carboxyl groups of key metabolites. An important assumption required for measuring de novo, anaplerotic glutamate synthesis is the degree to which oxaloacetate synthesized by pyruvate carboxylase equilibrates with fumarate in the astrocytes. Complete equilibration would produce equal labeling from $13CO_2$ or [1-13C] pyruvate into the 1 and 4 carbons of oxaloacetate and therefore appearance of only half the fixed CO_2 into glutamate. We have recently used focused microwave irradiation to sacrifice rats that have been perfused with [3-13C] glucose. We extracted the brains with perchloric acid and measured spectra of 13C labeled aspartate using a 500 MHz magnetic resonance spectrometer with attached cryoprobe. The aspartate, in equilibrium with oxaloacetate exhibits equal 13C labeling in carbons 1 and 4. The data demonstrate that oxaloacetate and symmetrical fumarate completely equilibrate. This information will provide more accurate modeling of brain metabolism by 13C-NMR spectroscopy.

P1-03-07

Metabolomic analysis of exogenous ethyl pyruvate and exogenous pyruvate for recovery of neonatal brain slices after oxidative stress

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Exogenous administration of ethyl pyruvate (EP) or pyruvate (pyr) can sometimes rescue cells about to die from injuries caused by

ischemia and/or oxidative stress. One perspective suggests that the rescue comes from EP and pyr being TCA Cycle substrates that bypass cytosolic glycolysis, which can be shut down by a pathological depletion of cytosolic NAD+ and NADH. A different perspective suggests that EP and pyr rescue primarily via radical scavenging by their carbonyl groups. We recently found EP was better than pyr in preserving ATP and reducing cellular injury after a 1 h exposure to 2 mM H_2O_2. That study compared rescue regimens of 2 mM glc plus one of the following: EP, pyr, the nonmetabolizable antioxidant PBN, and the combination EP with PBN. ATP was best preserved by the rescue combination 2mM glc, EP, and PBN. However, we also found that 2mM glc was suboptimal for sustaining slices, and concluded that new experiments with better rescue regimens wigh high glucose (10 mM) should be compared. Our hypothesis is that there is no difference between adding EP or a nonmetabolizable antioxidant during rescue. Using the same rodent brain slice model and the aforementioned perfusates after H_2O_2 oxidative stress, PCA extracts were studied with 31P/1H NMR. ATP ratios (values after rescue relative to pre-insult control) for the new experiments were the same for 10 mM glc, EP, and 10 mM glc, PBN. Adding EP provided the same protection as adding PBN. Multivariate analysis provided Scores and Loading plots that clearly demonstrated separate clusters for superior EP/pyr outcomes.

P1-03-08

Aspartate-glutamate homeostasis in cerebellar neurons changes when shifting from glucose to β-hydroxybutyrate as substrate

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Glucose is the primary energy substrate for the adult mammalian brain, but other substrates such as lactate and ketone bodies (β-hydroxybutyrate and acetoacetate) can be used under conditions of low glucose availability. Physiologically this is seen under starvation and in the suckling period in infants. Therapeutically, a ketogenic diet giving high levels of ketone bodies in plasma is used for the treatment of pharmaco-resistant epilepsy in children. The mechanism behind the effect of a ketogenic diet is still not understood but a change in amino acid homeostasis might be an important element. The present study was conducted to elucidate such an effect of β-hydroxybutyrate compared to glucose during synaptic activity. Primary cultures of mouse cerebellar neurons were superfused with buffer containing either [1, 6-13C] glucose or [2, 4-13C] β-hydroxybutyrate or combinations with only one of the substrates labeled. In neurons receiving only β-hydroxybutyrate as energy substrate, a change was observed in the aspartate-glutamate homeostasis towards a higher content of aspartate at the expense of glutamate. This change was reversed upon depolarization, analogous to what was found when glucose was present in combination with β-hydroxybutyrate. Conversion of β-hydroxybutyrate to acetyl-CoA is restricted to mitochondria. Thus, no NADH production occurs in the cytosol and malate-aspartate shuttling is therefore not required. The change in the aspartate-glutamate homeostasis in neurons receiving only β-hydroxybutyrate may therefore be due to decreased availability of NADH for the cytosolic malate dehydrogenase.

P1-03-09

Time-resolved measurement of rat brain glycogen absolute concentration *in vivo*

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13C-labeled glucose (13C-Glc) is administered to measure brain glycogen (glyc) *in vivo* by 13C NMR spectroscopy. Interpretation is thus potentially affected by changes in glyc turnover rates. We wanted to eliminate label turnover as a potential confound and to measure absolute [glyc] by establishing increased 13C Isotopic Enrichment using 13C-Glc at 'isotopic steady-state'. The infusion of 13C-Glc is performed at the IE of brain glyc. Thus, the observed changes in 13C glyc signal intensity will only reflect changes in absolute concentration. As brain glyc IE cannot be assessed *in vivo*, we estimated it from the measurement of N-acetyl-aspartate (NAA) IE, based on the fact that they have a similar turnover rate. After > 20 h of 13C-Glc ingestion, glyc IE measured *in vitro* was 2.2-fold that of NAA, as expected from the metabolic pathway of NAA synthesis from Glc. Then, in another group, after > 7 h of subsequent Glc infusion at an IE matching that estimated for glyc, we showed that the glyc IE was stable and similar to Glc IE suggesting complete turnover of the glyc molecule. Absolute [glyc] measured *in vivo* by NMR spectroscopy was similar to subsequent *in vitro* measurement. Finally, to test whether insulin administration results in net glyc synthesis, insulin was infused with 13C-Glc. The apparent stability of the signal of glyc was in agreement with previous biochemical quantification, suggesting a modest increase in total brain glyc content. These results suggest that insulin activates brain glyc turnover. In conclusion, a method to determine absolute [glyc] *in vivo* in a time-resolved manner provided an important insight into the role of glyc in the brain.

P1-03-10

Expression of pyruvate carboxylase in cultured rat oligodendroglial, microglial and ependymal cells

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The tricarboxylic acid (TCA) cycle plays a dual role in cellular metabolism. The TCA cycle is essential for the complete oxidation of the acetyl residue of acetyl-CoA and is a source of substances essential for several biochemical pathways. TCA cycle members withdrawn from the cycle are replenished by anaplerotic processes. A major role in brain anaplerosis is played by the mitochondrial enzyme pyruvate carboxylase (PC; EC 6.4.1.1), which catalyzes the ATP-dependent carboxylation of pyruvate to oxaloacetate. In mammals, the expression of PC is transcriptionally regulated in a tissue-specific manner via one of two distinct (distal and proximal) promoters. Thus, there are two different classes of PC mRNAs. In brain, the expression of PC has already been described and assigned to astrocytes. Since PC deficiency is associated with neurodegenerative phenomena, e.g. inadequate development of the corpus callosum and the lack of myelination, it can be hypothesized that PC may be expressed also in glial cells other than astrocytes. Therefore,

the expression of PC was investigated in cultured rat oligodendroglial, microglial and ependymal cells. PC mRNA, synthesized under the influence of the distal promoter, was detected by RT-PCR in all of these cell cultures. Furthermore, the expression of PC in cultured glial cells was studied, by immunoblotting and immunocytochemistry, using an antiserum generated in a rabbit. The results indicate that, in addition to astrocytes, also oligodendroglial, microglial and ependymal cells are capable of expressing PC and that the intermediary metabolism of these cells includes the anaplerotic function of PC.

P1-03-11

Decrease inorganic polyphosphate by PPX transfection in C6 rat glioma cell line could change its response to ischemia

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Inorganic polyphosphate (poly P), chains of hundreds of phosphate residues linked by 'high-energy' bonds as in ATP, has been conserved from prebiotic times in all cells. Poly P is essential for a wide variety of functions in bacteria and some lower eukaryotes. In our study, we explore the role of poly P in mammalian brain cells. A yeast polyphosphatase (PPX1), one of the main enzymes that degrades and releases orthophosphate (Pi) from the ends of poly P, was inserted into the chromosomes of C6 rat glioma cell line. The transfected cells responded differently to ischemia. Phosphorylation of signaling molecules, such as Erk1/2 and Akt, lasted for 8 h in the transfected cells under ischemia. Phosphorylation of these molecules lasted for only 4 h in control cells. In addition, control cells began to detach from the culture dish at 4 h ischemia and almost all cells detached at 16 h. Most of the transfected cells retracted their processes and became round but remained attaching to the culture dish at 8 h ischemia. At 16 h, the transfected cells were dead as confirmed by trypan blue staining; but these dead cells retained their intact appearance and remained attaching to the culture dish. Phosphate and energy are needed in the process of phosphorylation, change of cell morphology and programmed cell death. Our results suggest that decrease of poly P in C6 rat glioma cell line may slow down the metabolism and increase the resistance to ischemia.

P1-03-12

GAD_{65} is essential for the maintenance of GABA homeostasis

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The most abundant neurotransmitters in the brain are glutamate and γ-aminobutyric acid (GABA) for excitatory and inhibitory transmission, respectively. A number of pathological disorders might be explained by an altered metabolism of GABA. The biosynthesis of GABA is catalyzed by the enzyme glutamate decarboxylase (GAD) and their existence as two independently regulated genes, known as GAD65 and GAD67, remains unlcear. The metabolic implication on brain amino acid homeostasis caused by the absence of GAD65 was investigated by incorporating [1-13C] glucose and [1, 2-13C] acetate. into amino acids in the brains of adult GAD65 knockout mice and wild type mice. Acetate is taken up exclusively into the astrocytic compartment, whereas acetyl coenzyme A generated from glucose is predominantly metabolized in neurons. The incorporation of 13C-label into metabolites was determined using 13C NMR spectroscopy. While, the total amounts of metabolites were assessed using 1H NMR spectrocopy and remained unaltered. In contrast, the amount of labelled amino acids, and thus synthesis and turnover rates, were significantly lower in GAD65 knockout mice compared to wild type, implying that the general energy and amino acid metabolism are affected by the absence of GAD65. In addition, the effect of the full GABAA-receptor agonist isoguvacine (IGU) on the frequency of spontaneous epileptiform discharges (SED) in GAD65 knockout mice was evaluated. Increasing concentrations of IGU led to a concentration dependent inhibition of the SEDs. The inhibitory effect was lower for GAD65 knockout mice and might be explained by a lower concentration of endogenous GABA or an altered expression or functionality of GABAA-receptors.

P1-03-13

Redox dependence and pyruvate compartmentation during simultaneous metabolism of ^{13}C glucose and ^{13}C monocarboxylates in the adult rat brain as detected by (^{13}C, ^{2}H) NMR spectroscopy

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Pyruvate plays a central role in cerebral metabolism, integrating glycolytic and oxidative responses under physiological and pathological conditions. A single pool of intracellular pyruvate has been previously assumed in most *in vivo* 13C NMR studies of cerebral metabolism. However, intracellular pyruvate compartmentation has been demonstrated in primary cultures of neurons and astrocytes, as well as in C6 glioma cells. In this work, we describe the presence of two slowly exchanging pools of cytosolic pyruvate in different regions of the adult brain of rats bearing C6 gliomas. C6 gliomas were induced in anesthetized male Wistar rats by injection. Ten days before the infusion, one group received 50% D_2O in the drinking water and one did not. Within the fourth week after injection, from each group half the rats were infused with a solution of 0.2 M of (1–13C) glucose and 0.4 M of (2-13C) pyruvate, while the other half received 0.4 M of (U-13C3) lactate instead of pyruvate. Extracts from the different regions of every brain were analyzed by high-resolution 13C NMR spectroscopy to determine the relative proportions of 13C lactate isotopomers, derived from the 13C glucose or 13C monocarboxylates. The relative production of lactate derived from (1–13C) glucose to that derived from monocarboxylates decreased in the order contralateral > ipsilateral > tumour. These results suggest that the different glycolytic rates obtained in the contralateral, ipsilateral and tumourous tissues are determined by the oxygenation or redox state of the tissue. The different deuteration patterns observed in (2–13C) and (3–13C) lactate reveal, for the first time to our knowledge, the intracellular compartmentation of the pyruvate pool in the adult brain *in vivo*.

Our results indicate that redox state and pyruvate compartmentation modulate cerebral glycolytic flow *in vivo*.

P1-03-14

Metabolic profiles of the brains of Zucker Diabetic Fatty, Zucker Obese and Sprague-Dawley rats and the involvement of glycogen metabolism

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The number of diabetes sufferes is increasing especially in the Western world and is characterized by insulin deficiency which consequently alters carbohydrate metabolism and impairs the glucose regulatory system. Liver glycogen is essential in controlling blood glucose levels. Glycogen, however, also exists in the brain, and little is known about its metabolism in the diabetic state. We investigated brain energy and neurotransmitter metabolism in two diabetes animal models and a control model. A glycogen phosphorylase inhibitor (CP-316,819) was used to elucidate the role of glycogen metabolism in Zucker Diabetic Fatty (ZDF), Zucker Obese (ZO) and Sprague-Dawley (SprD) rats. Brain metabolism was studied by injection of [1-13C] glucose, 24 h after treatment with vehicle or CP-316,819. After 30 min, LC/MS determination of 13C labeling of glycogen, lactate, glutamine, glutamate and GABA was conducted. In all rat strains, labeling in glutamate was lower in the hippocampus compared to cortex and cerebellum. GABA was markedly higher in cerebellum. Inhibition of glycogen metabolism by CP-316,819 increased blood glucose labeling in SprD and ZO rats, whereas it was unchanged in ZDF rats. The 13C labeling of glutamate and GABA was not affected by inhibition of glycogen metabolism in SprD rats. Interestingly, normalized 13C-labeling of glutamate and GABA was decreased in ZO rats and increased in ZDF rats when glycogen metabolism was inhibited. In conclusion, similar regional brain labeling patterns exist between the animal strains, whereas the involvement of glycogen metabolism in brain energy and neurotransmitter metabolism differs markedly between SprD, ZO and ZDF rats. Further metabolites are currently being analyzed.

P1-03-15

NMR determination of brain glycogen turnover with internal labelling monitoring

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Glycogen is an important energy store in the brain and has been implicated in hypoglycaemia. To further characterise its role, it is important to determine its turnover time. The only *in vivo* method available is based on the incorporation of 13C-labeled glucose into brain glycogen and its detection with localized NMR. It is difficult to differentiate the increases in glycogen signal due to changes in 13C-isotopic enrichment. In this study we present an approach based on the 'pre-labelled' 13C1 position of glycogen and an acute infusion of C1. Three male Sprague-Dawley rats were fed 1-13C glucose dissolved in water for 24 h. Following preparation and placement in the 9.4 T scanner (Varian/Magnex Scientific, Yarnton, UK), glycogen C1 IE was determined *in vivo*. Then, 1, 6-13C2 glucose at this IE was infused for the next 7 h while glycaemia was kept at 10 mM. A new spectroscopic imaging technique was implemented to simultaneously measure both brain C1- and C6-glycogen every hour. The glycogen C6 time course was fitted with an exponential function to obtain the turnover time. The stability of the glycaemia was evident from the stable brain glucose C1 signal. The brain glucose C6 signal exhibited a step increase as expected. Glycogen C6 on the other hand gradually increased and from we obtained an estimated turnover time of 8 ± 4 h, within the literature value of 10 h. In conclusion, it is feasible to image brain glycogen metabolism *in vivo* using 13C NMR and derive net concentration changes along with unidirectional synthesis of brain glycogen.

P1-03-16

Modifications of brain activity are associated with changes in aerobic glycolysis

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Task performance evokes dramatic increase in cerebral blood flow and glucose utilization but much less change in oxygen utilization in activated brain regions. This lack of proportionality, demonstrated initially with positron emission tomography (PET), suggests increase in aerobic glycolysis and constitutes the physiological basis of blood oxygen level dependent (BOLD) magnetic resonance signal. In this study, we have assessed regional PET measures of brain aerobic glycolysis during physiological stimulation in comparison to the resting state. Eight healthy young adults were studied at rest and during performance of the Word Stem Completion Task (WSCT), CBF was measured with PET using the intravenous bolus [15O] H_2O method. Images of aerobic glycolysis expressed as glycolytic index (GI) was generated corresponding to the amount of glucose utilization that differs voxel-by-voxel from that predicted by oxygen utilization. WSCT performance induced CBF increases and decreases in a distribution as expected. CBF decreased in response to the WSCT in 'default network' regions including posterior cingulate cortex, ventromedial and dorsal prefrontal cortex, lateral parietal cortex, and lateral temporal cortex ($p < 0.01$, two-tailed paired *t*-test). CBF increased in regions known to respond positively to the WSCT including visual and motor cortex, ($p < 0.001$, two-tailed paired *t*-test). CBF and changes in these regions were accompanied by corresponding changes in GI and CMRGlc ($p < 0.01$, two-tailed paired *t*-test), but not in CMRO2. We conclude that modifications in brain activity commonly detected with modern functional neuroimaging methods are associated with the changes in aerobic glycolysis.

P1-03-17

Traumatic brain injury significantly alters hippocampal energy metabolism

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Every 20 s an individual in the United States suffers a traumatic brain injury (TBI), which is the primary cause of death and disability in young adults and children. Changes in cellular energy metabolism associated with TBI have yet to be thoroughly examined, although changes in lactate and pyruvate levels meaured by dialysis have been reported. Currently there is no treatment or therapy for the underlying causes of neurological dysfunction and the clinical focus on management is treatment of associated symptoms. Therefore, our laboratory has examined underlying mechanisms which cause disruptions in the delicate balance of cellular metabolism and inhibitory and excitatory synaptic tranmission in the hippocampus. Using a lateral fluid percussion injury model in mice, we examined hippocampal slices from sham and TBI mice 7 days following injury. Pyruvate dehydrogenase, exclusively expressed in glial cells, demonstrated decreased expression after TBI. Additionally, pyruvate carboxylase (PC) expression was reduced after injury when compared to sham. Importantly, PC is clearly expressed in both astrocytes and neurons, which suggests that both cellular populations are capable of directly converting oxaloacetate to pyruvate. Finally, extracellular recordings in hippocampal area CA1 of sham mice show that direct application of lactate or pyruvate causes reduced excitability which is not observed in injured mice. The lack of deleterious affect in the injured mice is likely due to injury-induced changes in expression of glucose metabolizing enzymes suggesting an improved abilitity to anaerobically metabolize glucose. The results described here implicate a multi-faceted response to TBI. The changes in energy metabolizing enzymes suggest a significant shift in normal cellular metabolism after injury.

P1-03-18

Fos expression and nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d) reactivity in rat brain and hormone changes after electrical stimulation of medial amygdala

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Recently, the posterodorsal amygdala (PDA) has been suggested as a possible effective site to produce hyperphagia and obesity in female rats. Our pre-experiments showed that unilateral PDA electrical stimulation decreased the food intake of female rats with 23 h fasting. The possible mechanisms of PDA regulating food intake were explored through testing Fos expression, NADPH-d staining, and the changes of correlated hormones. After electrical stimulation Fos–like immunoreactivity (FLI) and NADPH-d were assessed in several regions of forebrain and brain stem. FLI was detectable in thalamus, hypothalamus, amygdala, parabrachial nuclei (PBN), nucleus of the solitary tract (NST), and area postrema (AP), both in stimulated and control groups. However, electrical stimulation significantly increased the c-fos expression in arcuate nucleus (Arc), central nucleus of amygdalA), external subnuclei of the lateral part of PBN, AP, and the caudal part of NST. A few Fos/NADPH-d double-labeled neurons were seen in the dorsal of the lateral part of PBN and the intermediate NST. Levels of leptin, insulin, corticosterone, corticotrophin releasing hormone, and adrenocorticotropic hormone in the serum, and CRH concentration in the hypothalamus and the left amygdale were examined by radioimmunoassay after electrical stimulation. Compared with control group electrical stimulation significantly elevated serum leptin level. There was no significant difference between groups for any of the other hormones. These results suggest that CeA, Arc, lateral part of PBN, caudal NST and AP participate in the regulation of PDA on food intake, without nitric oxide involved. PDA may mediate feeding behavioa via modulating circulating leptin.

P1-03-19

NMDA receptor hypofunction induced by MK-801: amino acid and monoamine homeostasis

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The noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist MK-801 has been widely used as an animal model of schizophrenia. Glutamate receptor antagonists have neurotoxic and psychotogenic properties in addition to their neuroprotective potential during excessive glutamate release. We have analysed the effects of MK-801 on levels of amino acids and monoamines, including glutamate, glutamine, GABA, dopamine and serotonine in rat brain. Rats were given a subanesthetic dose of MK-801 or saline. Thirty minutes later an injection containing [1-13C] glucose was given, followed by microwave fixation of the brain 15 min later. During the experimental period all rats showed hyperlocomotion, ataxia, abducted hindlimbs, flat body posture and stereotyped behavior such as head weaving. In an earlier study, analyses of extracts from cingulate plus retrosplenial cortex or temporal lobe were performed using high performance liquid chromatography (HPLC), 13C and 1H nuclear magnetic resonance spectroscopy. Hypofunction of the NMDA receptor induced similar changes in both brain areas investigated, however, the changes were most pronounced in the temporal lobe. The two regions analysed are very heterogeneous and included several defined subregions. Here, striatum, nucleus accumbens, amygdala, thalamus, hippocampus, frontal cortex and temporal cortex were dissected from the microwave fixated brains and after extraction, amino acids and monoamines were analysed with HPLC and gas chromatography mass spectrometry in all brain areas. 13C and 1H NMR spectroscopy was performed on larger areas. Glutamatergic hypofunction was confirmed in the frontal cortex. GABAergic metabolism was decreased as well. The analyses of monoamines points toward altered monoamine oxidase activity in striatum and nucleus accumbens.

P1-03-20

Investigation blood flow velocity change of cerebral surface arterioles at spatial resolution of micrometer scale by ODT technique

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Optical Doppler Tomography (ODT) has emerged recently as a potential imaging technology to study cerebral microcirculation. Compared with the laser-Doppler flowmetry technique which can just measure the blood flow response of millimeter scale vessels, ODT can provide in real-time high spatial resolution imaging of biological tissues at micrometer scale in a noninvasive way. In this study, we applied ODT to investigate the blood flow velocity change of cerebral surface arterioles in SD (Sprague-Dawley) rats by administration of Noradrenalin and electrical stimulation. Before the ODT scanning experiments, rats were anesthetized, then the head was secured in a stereotaxic frame, a midline scalp incision is made, and a 3 × 5 mm area of skull is thinned, leaving a thin translucent cranial plate. Electrical stimulation: two subdermal needle electrodes were inserted into the dorsal hind paw, stimulation frequency was 5 Hz, stimulation current amplitude was 1 mA; Drug administration: Before drug experiments, neck vein intubatton is made, then Noradrenalin (210 μg/kg) is injected by injector during experiments. The results showed significant differences in blood flow velocity between experimental groups and control groups, both under electrical stimulation and drug administration. Compared with electrical stimulation result, drug administration has greater influences. In conclusion we demonstrate the feasibility of ODT technique in application of cerebral microcirculation study, which will be helpful to understand the mechanism such as the cerebral-vessel diseases, the neurovascular coupling, it may also have a potential in clinical diagnosis.

P1-03-21

Biochemical evidence for a significant blood-brain barrier for glucose in hypothalamus using the reversible Michaelis–Menten model

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Previous studies into the regulation of brain glucose metabolism and transport across the BBB have shown a linearity of brain glucose concentrations at given plasma glucose concentrations. This study extends these measurements to the hypothalamus which has been proposed to have at least in part an incomplete BBB, where higher brain glucose content is expected. Euglycemic, insulin-induced hypoglycemic and high glucose/somatostatin-induced hyperglycemic rats underwent overnight fasting, α-chloralose anesthesia and plasma glucose sampling prior to focused microwave fixation. Detection of tissue glucose and release of glucose from glycogen by glucosidase enzyme from hypothalamus and cortex (control) was based on the reaction of glucose oxidase with D-glucose to form D-gluconolactone and H_2O_2, the later product then reacting with peroxidase substrate in the presence of HRP to generate the red-fluorescent oxidation product resorufin in a linear fashion between < 2 and 125 μM with a precision of < 2%. Glycogen content was approximately 90% higher in hypothalamus than in cortex; it varied almost in a linear fashion with glucose content, and after 2 h at < 1.5 mM plasma glucose was approximately 2 μmol/g, or 80% higher than in cortex. The higher glycogen level at rest and following hypoglycemia implies a higher neuroprotective potential of glycogen in hypothalamus. The relationship between hypothalamic and plasma glucose was linear from < 1.5 to 20 mmol/L plasma glucose. Furthermore, the hypothalamic brain glucose concentration was comparable to that in cortex. These results suggest that the BBB is the rate limiting step for transport also in hypothalamus.

P2-01-01

Age related neurodegeneration with reference to senile dementia of Alzheimer's type – role of an Ayurvedic formulation

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Senile Dementia of Alzheimer's Type (SDAT) is considered the most common cause of dementia. Ten percent of population, over the age of 70 years has a significant memory loss. The etiological factor of SDAT ranges from oxidative injury, autoimmune complexes, and inflammatory factors to platelet aggregations, from syntheses and degradation of amyloid proteins to neurotransmitters, and from cerebrovascular pathology to neuron–neuron degeneration. Ayurveda is a traditional system of medicine which recommends a variety of health care modalities including medicinal plants that can regulate and slow down the biological aging. In view of the above the hydro-alcoholic extract of Ayurvedic plants Hippophae rhamnoides (leaves and fruits) and Bacopa monnieri (whole plant) were orally administered for 1 year to 49 diagnosed SDAT cases with age range of 62–75 years of both sexes. Thirty-eight cases received placebo treatment and provided the data to compare the results. It was noticed that test formulation modulates the cholinergic, serotonergic, noradrenergic and dopaminergic brain chemistry, with the result the further loss of memory, attention and other cognitive functions were checked and improved. As synergism, the test drug exhibited anti-oxidant and immunomodulatory effects. Before commencing clinical trial of test drug the safety and efficacy profile was established in animal models. The ethical approval was sought and informed written consent of the subjects was taken. It is proposed that the test drug is a better remedial measure for the prevention and management of age related neurodegeneration including SDAT.

P2-01-02

Preliminary evidence for potential neurological damage following nanoparticle exposure

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Recently, there has been increasing concern that nano-sized particulate matter can be translocated into the brain and may be associated with neurodegenerative diseases. Central nervous system

(CNS) is the potential susceptible target of inhaled nanoparticles, however, little is yet known what kind of pathological changes will happen after nanoparticles reach the brain. In present study, we firstly focused on the trafficking of TiO_2 and Cu nanoparticles into murine brain and further biochemical changes after intranasal inhalation. Synchrotron radiation X-ray fluorescence analysis (SRXRF) and inductively coupled plasma mass spectrometry (ICP-MS) were therefore used to determine the qualitative and quantitative contents of titanium and copper in the brain sections at different position. Then, the pathological examination of brain tissue, oxidative stress-mediated responses, and metabolisms of neurochemicals in the brain of exposed mice were also analyzed. The obvious morphological changes of hippocampal neurons and GFAP-positive astrocytes were observed. Oxidative stress occurred obviously in whole brain of exposed mice such as lipid peroxidation and protein oxidation, as well as upregulated activities of catalase and lactate dehydrogenase and the excessive release of neurotransmitter (including glutamic acid, nitric oxide). Our findings provide the preliminary evidence that nasal instilled nanoparticles could be translocated into the central nervous system and cause neurological damage, and the hippocampus would be the main target within brain. We suggest that nanoparticles might translocate from nasal mucosa via olfactory nerve to brain where they induce oxidative stress and stimulate neurotransmitter release.

P2-01-03

Research on the mechanism of abnormal serum triglyceride caused by antipsychotic agent in patients with senile dementia

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The risk factors of hypertriglyceridemia, an abnormal character in patients with senile dementia, were studied after receiving antipsychotic agent therapy. Enzyme methods were used to monitor the serum levels of triglyceride (TG). High-density lipoprotein cholesterol (HDL-C), total cholesterol (TC) level in 88 patients' with senile dementia (38 patients with Alzheimer's disease AD, and 50 patients with vascular dementia VD) and 90 normal controls were analyzed. The levels of TG increased evidently compared to the normal control group ($p < 0.01$). However, the levels of HDL-C in serum decreased markedly compared to the normal control group ($p < 0.01$). High TG and low level HDL-C are the most significant lipid abnormal characters of senile dementia. High TG and low HDL-C due to prolong use of antipsychotic agents can lead to arteriosclerosis, especially in AD and VD which can deteriorate cognitive function. The negative correction of high TG and low HDL-C is a biological risk factor of ischemic diseases of the heart and brain.

P2-01-04

MCP-1 activates PI3K-Akt signaling pathway in the primary cultured rat cortical cells

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Chemokines are originally described in the immune system, where they promote the recruitment of leukocytes into inflammatory sites. Chemokines and their receptors have also been recognized as key signaling molecules in the central nervous system (CNS), participating in many neuroinflammatory processes as well as in regulatory functions of the normal brain. Recent evidence indicates that the expression of monocyte chemoattractant protein-1 (MCP-1), a CC chemokine, is significantly upregulated or dysregulated in a variety of acute and chronic neurodegenerative CNS disorders. However, relatively little is known about functional roles of MCP-1 in neurons or the biological consequences of its receptor activation. In the present study, we identified the expression of CCR2, the major receptor of MCP-1, in primary cultured rat cortical cells and investigated its intracellular signaling pathways using Western blot analyses. We found that CCL2 induced phosphorylation of phosphatidylinositol 3-kinase (PI3K) and its downstream kinase Akt in time- and concentration-dependent manners. Akt was phosphorylated at Ser 473 and selectively inhibited by the PI3K inhibitor LY294002. However, pretreatment with pertussis toxin, a toxin that inactivates Gi/Go protein by ADP ribosylation, had no significant effect on the level of MCP-1-induced phosphorylation. These findings demonstrating the involvement of PI3K-Akt pathways in the MCP-1 signaling in the cultured rat cortical neurons may be central to an understanding of the functional roles of MCP-1 in the CNS under physiological or pathological conditions. Potential role of PI3K-Akt activation by MCP-1 in cell survival is discussed.

P2-01-05

Complex trait analysis of susceptibility to paraquat-induced Parkinsonism

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Administration of the herbicide Paraquat (PQ) causes a well defined degeneration of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNpc). Like the dopaminergic toxin MPTP, the loss of SNpc DA neurons shows strain differences: C57BL/6 (C57) mice lose approximately 50% of their SNpc DA cell population, whereas Swiss-Webster mice (SWR) lose only 10%. Analysis of reciprocal F1 intercrosses between these strains suggests that higher susceptibility to PQ is inherited as a dominant trait from the C57 parental strain. One method for identifying genes that confer this PQ sensitivity is the identification of quantitative trait loci (QTLs), we have previously used this method to identify a QTL for MPTP sensitivity and subsequently identified glutathione S-transferase pi (GSTp) as a MPTP-sensitivity gene. In order to determine if the toxicity of PQ shared the same mechanism as MPTP we genotyped a set of 65 microsatellite markers distributed across the genome by gel electrophoresis in a set of 66 backcross progeny. Markers with both high positive and negative correlations ($r > 0.40$) to surviving numbers of SNpc cells were detected. This suggests that variation in sensitivity to PQ may involve two or more loci located on mCHr5 centered around D5Mit338 and mChr14 centered around D14Mit206. The localization of these loci is different from that identified for MPTP and suggests different genes are different in regard to PQ neurotoxicity. The confirmed identification and ultimate cloning of QTLs that that underlie sensitivity to PQ may lead to a better understanding of the molecular basis of human Parkinson's disease.

P2-01-06

Effects of tau protein concentrations in cerebrospinal fluid of patients with vascular dementia

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The diagnostics of vascular dementia was explored by measuring the level of tau protein in cerebrospinal fluid. Level of tau protein in cerebrospinal fluid measured in 48 patients (65.2 ± 13.8) and 45 control groups (62.7 ± 12.6) by enzyme-linked immunosorbent assay double sandwich method (ABC-ELISA). The level of tau protein increased significantly in cerebrospinal fluid of vascular dementia (152.36 ± 87.66) pg/ml patients, as compared with those in control groups (74.16 ± 22.81) pg/ml ($P < 0.01$). Measuring the level of tau protein in cerebrospinal fluid might be an important parameter for clinical diagnosis in patients with vascular dementia.

P2-01-07

The implication of oxidative stress in a rat model of Parkinson's disease

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Parkinson's disease (PD) stems from the loss of dopamine caused by the degeneration of the dopaminergic neurons of the substantia nigra. Current theories suggest that reactive oxygen species are involved in some capacity early in the disease process. The administration of 6-hydroxydopamine (6-OHDA) into the brain of the rat produces a well-established model of PD. Many investigators have demonstrated that 6-OHDA induces oxidative stress which can lead to the induction of apoptosis and cellular loss. The purpose of the present study was to determine the development of oxidative stress that is generated in a substantia nigra (SN) and ventral tegmental area (VTA) 6-OHDA lesion model of PD through assessing the antioxidant enzymes activities in the temporal and frontal lobes homogenates. Male Wistar aged rats were used. Biochemical estimations were performed by determination of superoxid dismutase (SOD), glutathione peroxidase (GPX) and malondialdehyde (MDA) activities. Lesioning of substantia nigra and ventral tegmental area with a low dose of 6-OHDA induced significant reduction in SOD and GPX specific activities, but also a non-significant reduction of MDA concentration in the temporal lobe rather than in the frontal lobe homogenates, comparative with sham-operated control group. Also, the role of the substantia nigra is more prominent than that of the ventral tegmental area. Our results support that oxidative stress plays a role in the damage produced by substantia nigra and ventral tegmental area injection of 6-OHDA, and that indices of oxidative stress could potentially be important markers for evaluating therapeutic strategies and their effects on 6-OHDA-induced dopaminergic neurotoxicity.

P2-01-08

NAD^+ synthesis and a 16 amino acid N-terminal sequence on the slow Wallerian degeneration protein (Wld^S) synergise to confer axon protection

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The slow Wallerian degeneration (Wld^S) protein, formed by the N-terminal 70 amino acids (N70) of multiubiquitination factor Ube4b and full length NAD^+ synthesising enzyme Nmnat1, greatly delays acute axon degeneration after injury, termed Wallerian degeneration, and alleviates axon pathology in some models of neurodegenerative disorders. Highly overexpressed Nmnat1 preserves injured neurites *in vitro*, but Nmnat1 without N70 fails to protect axons in mice. The AAA-ATPase valosin-containing protein (VCP/p97) binds the N-terminal 16 amino acids (N16) of Wld^S, influencing the intracellular locations of both Wld^S and VCP/p97. Removing N16 abolishes axon protection in transgenic mice but replacing it with an ataxin-3-derived VCP binding motif restores the protective phenotype. N16 is not sufficient for phenotype because injured axons in transgenic mice expressing an enzyme-dead variant of Wld^S degenerate rapidly. Moreover, we found that reducing NAD^+ levels in Wld^S primary neuronal cultures weakens the axon protection phenotype. However, Nmnat1 heterozygote knock-out mice, where enzyme activity is reduced, have a wild-type rate of Wallerian degeneration. Therefore, Nmnat1 activity and increased NAD^+ levels are able to delay Wallerian degeneration as long as Nmnat1 is associated with other sequences, but reducing NAD^+ from wild-type levels does not accelerate it. We hypothesise that Nmnat1 activity synergises with the N16 domain of Wld^S protein to confer neuroprotection, which probably redistributes Nmnat1 to a particular subcellular localisation.

P2-01-09

Efflux of amyloid-beta$_{1\text{-}40}$ via perivascular routes from inferior colliculus of normal rats

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Amyloid deposits are located in interstitial and perivascular spaces in brains of Alzheimer patients and animals used as models for Alzheimer's disease but it is not known whether the perivascular pathway is a normal route for clearance of amyloid-beta (Aβ) from brain. The distribution of Aβ was assayed by immunohistochemistry and fluorescence microscopy 60–90 min after intracerebral microinfusion or microinjection of unlabeled $Aβ_{1\text{-}40}$ or Cy-5-labeled Aβ into the inferior colliculus of 18 rats. The distributions of labeled and unlabeled Aβ were similar, with highest labeling in tissue surrounding the injection site, prominent labeling of meninges surrounding the inferior colliculus, and perivascular labeling of blood vessels at the base of the brain and under the olfactory bulb. Background labeling for the fluorescent tracer was very low and pre-treatment of the primary antibody with blocking peptide or omission of the primary antibody also yielded low background labeling. These results show diffusion of exogenous Aβ from a point source into perivascular pathways that drain into lymph nodes

as a normal route for clearance of extracellular Aβ in adult rat brain. Thus, continuous clearance of Aβ from interstitial fluid derived from a substantial tissue volume could converge to a common perivascular pathway producing cerebral amyloid angiopathy, which is unlikely to arise from only local production of amyloid protein. Partial blockade of perivascular fluid flow by amyloid deposits could contribute to the pathophysiology of Alzheimer's disease by interfering with clearance of Aβ and of important metabolic by-products, such as lactate.

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P2-01-10

Age consistent hyperhomocysteinemia and risk of neurodegeneration – benefits of hippophae rhamnoides

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Elevated plasma Homocysteine (Hcy) has found to play key role in neurodegenerative disorders, particularly Alzheimer's disease and Parkinson's disease. High concentrations of Hcy acts as a neuro-toxin, causes oxidative stress and glutamatergic excito-toxicity suggests a risk for neuropsychiatric disorders such as schizophrenia, depression etc. The high prevalence of hyperhomocysteinemia in elderly population is warranted for a safer treatment strategy to prevent them from future consequences of neurodegeneration. In view of the above 82 elderly volunteers of both sexes with age range of 60-72 years were selected showing high Hcy levels (> 15μmol/L). Under study 39 elderly were kept on placebo and 43 elderly were treated with hydro-alcoholic extract of Hippophae rhamnoides for 6 months. Hcy and CRP were measured by ELISA kit method and C-reactive protein (CRP) by kit for quantitative nephelometric determination of CRP in human serum or plasma. It is observed that Hcy level enhances with advancing age and females exhibited higher Hcy level than male subjects. Results revealed that initial high Hcy along with high inflammatory markers CRP and TNF-α declined significantly following test drug treatment. Placebo did not show any beneficial change in the parameters. It is proposed that Hippophae rhamnoides is a better remedial measure in the prevention and management of hyperhomocysteinemia reducing involved inflammatory process, oxidative injury among the elderly subjects, where the synthetic chemical interventions are restricted and thus the risk of various neuropsychitric disorders including vascular complications can be prevented/minimized to a great extent.

P2-01-11

Transduction of Uch-L1 protein reverses synaptic and cognitive impairments in a mouse model of Alzheimer's disease

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The neuronal ubiquitin-proteasomal system is linked to the pathogenesis of sporadic Alzheimer's disease (AD). In this study, we demonstrated that the enzymatic activity of the memory-related molecule, Uch-L1, is reduced in the *APP/PS1* mouse model of the disease. LDN-57444, a specific Uch-L1 inhibitor, markedly reduced both synaptic and cognitive functions. Following treatment with inhibitor, levels of basal synaptic transmission (BST) and long term potentiation (LTP) were ~39% and 73% of control vehicle treated slices, respectively. Intraperitoneal injection of LDN-57444 reduced the mouse freezing time during contextual learning to ~75% and 70% of control-vehicle injected mice. Transduction of Uch-L1 protein through a recently developed technology in which an 11-amino acid transduction domain of HIV-transactivator protein (TAT) is fused with the Uch-L1 protein re-established normal synaptic function both in hippocampal slices treated with oligomeric Aβ and in *APP/PS1* animals. LTP was equal to ~94% of vehicle-treated slices following perfusion with TAT-HA-Uch-L1 in the presence of 200 nM Aβ (Aβ alone treated slices showed values equal to ~59%). LTP was equal to ~93% of WT littermates in slices from APP/PS1 mice perfused with TAT-HA-Uch-L1. Moreover, we found that a single injection of TAT-HA-Uch-L1 re-established a normal freezing time during extinction of contextual learning in *APP/PS1* mice. Finally, we demonstrated that the beneficial effect of the Uch-L1 fusion protein is linked to restoration of normal levels of PKA regulatory subunit II leading to normal kinase activity and CREB phosphorylation. Thus, drugs that enhance the activity of the proteasome system might have a beneficial effect in AD.

P2-01-12

A study on the degradation pathway of Aph-1 and Nicastrin, two critical components of γ-secretase complex

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The γ-secretase catalyzes intramembrane proteolysis of various type I transmembrane proteins that play a key role in the pathogenesis of Alzheimer's disease (AD). Presenilins (PS), presenilin enhancer-2 (PEN-2), Nicastrin (NCT) and anterior pharynx defective-1(Aph-1) are the essential components of the γ-secretase complex. Abnormality of the ubiquitin-proteasome pathway has been implicated in neurodegenerative disorders. The turnovers of presenilins and PEN-2 are reported to be regulated by the ubiquitin proteasome pathway. The degradation pathway of another two components, Aph-1 and NCT are still unknown. Western blot, pulse-chase metabolic labeling technique, immunoprecipitation, double immuno-fluoresecnt staining, and ELISA assay, combined with proteasomal and lysosomal inhibition were all used to investigate the Aph-1 and Nicastrin expression levels in neuronal and non-neuronal cells. By treating cells with proteasome specific inhibitors, we found that expression of endogenous and exogenous Aph-1 was significantly increased both in neuronal and non-neuronal cells. While treatment of cells with either proteasomal or lysosomal inhibitors can significantly increase both endogenous and exogenous NCT in various cell lines. Results also revealed that both Aph-1 and NCT are colocalized with ubiquitin in cells. NCT accumulates in the ER and Golgi apparatus after proteasomal inhibition and lysosomal inhibition leads to the accumulation of NCT in lysosomal apparatus. The turnover of newly-synthesized radiolabeled Aph-1 protein was blocked by proteasomal inhibitor, while both proteasomal and lysosomal inhibition causes a prolonged half-life of NCT in cells. Our study demonstrates that the degradation of Aph-1 protein is

mediated by the ubiquitin-proteasome pathway, while the degradation of NCT involves both the proteasome and lysosome.

P2-01-13

Studies on dCtr1C function and its role in fly neurodegenerative models

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Copper is essential in important physiological roles and in numerous reactions critical to cellular physiology. There is a close relationship between copper dyshomeostasis and neurodegeneration, and copper mediated free radicals are generally considered one of the causes of nerve damage. Comparative studies have been performed for Ctr1A and Ctr1B copper transporters in the *Drosophilia* genome. Our results demonstrated that flies overexpressing dCtr1C in the brain (elav-Gal4 driven) and whole body (actin-Gal4 driven) would developmentally arrest and die at the second or third instar larval stage. Adding EDTA to their diet could not rescue the lethal phenotype, but adding the copper specific chelator BCS did. However, overexpressing dCtr1C in fly eyes (GMR-Gal4), wing (A9-Gal4) and heart (Timan-Gal4) resulted in normal flies. These results indicated that dCtr1C is related to copper uptake and the lethal phenotype of dCtr1C overexpression in brain and whole body depends on the dosage of dCtr1C expressed; possibly due mostly to copper poisoning in the brain resulting in nerve degeneration and death. We found that elav-Gal4 driven dCtr1C RNAi transgenic flies showed more resistance to oxidative stress, and dCtr1C knock down in fly brain could prolong the life-span of Aβ42 flies. Preliminary histological analysis of fly frontal brain sections also showed that dCtr1C RNAi in brain could reduce the number of vacuoles in AD flies. It is possible that knocking down dCtr1C reduces copper levels in fly brain, decreasing copper induced free radical reactions and accumulation of the Aβ42 monomer, thus delaying disease onset and extending the AD flies' life-span.

P2-01-14

In vivo assessment of oxidative stress in human brain in aging and Alzheimer's disease

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Glutathione (GSH) is a major antioxidant in the human brain and is suggested to be a sensitive indicator of oxidative stress in normal aging and the neurodegenerative diseases including Alzheimer's disease (AD). GSH levels in the human brain have been investigated mostly from brain specimens obtained from autopsy or biopsy due to lack of non-invasive measurement techniques. We measured GSH levels in human brain in aging and AD using a multiple quantum filtered spectroscopic imaging technique of GSH, which allowed a simultaneous measurement of GSH and creatine for reliable quantification of GSH. All measurements were performed using a 3 T MR system. A spectroscopic imaging slice was placed axially to cover fronto-parietal lobes of the brain. GSH concentration in the fronto-parietal region of elderly healthy human subjects was 11% lower than that of young subjects ($p = 0.02$). GSH concentration was further reduced in AD subjects by 18% compared with young subjects ($p < 0.001$). The GSH concentration difference between the elderly and AD groups was statistically significant ($p = 0.03$). In summary, we have demonstrated the reduction in GSH concentration in aging and AD in the human brain *in vivo*. These results are consistent with the effect of increased oxidative stress in the aging and AD brain. This unique capability of measuring GSH in the living human brain will allow us to monitor the effect of aging and progression of AD as well as the effect of interventions directed at antioxidant treatment in a longitudinal manner.

P2-01-15

In search for early electrophysiological indicators for Alzheimer's disease in APP695SWE transgenic mice

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Alzheimer's disease (AD) is the most common neurodegenerative disease in the elderly. Access to early disease indicators in experimental models for AD is essential for developing effective treatments. Here we used electrophysiological methods to study synaptic transmission and plasticity in hippocampal slices from 8-10 months old transgenic mice that overexpress the human APP695SWE mutant form of amyloid precursor protein, aiming to detect changes at an early stage of AD. Our study showed that both baseline synaptic transmission (BST) and paired-pulse facilitation (PPF) as well as population spike firing threshold were unchanged in APP695SWE transgenic mice as compared to wild-type controls. Both early and late phases of long-term potentiation (LTP) were also unaffected as revealed by recordings lasting up to 6 h after induction. Still, an extended analysis of BST revealed a hitherto unnoticed prolongation of EPSP waveform in the mutant mice, present for EPSPs above a certain minimal size. An opposite change, shortening of EPSP waveform, occurred in normal mice after application of the broad spectrum muscarinic agonist carbachol. Our data suggest that in APP695SWE mice up to 10 months, synaptic function and plasticity might not be impaired to the extent that changes are readily detected by standard electrophysiological methods, or the changes could be masked by compensatory mechanisms. However, the observed EPSP waveform prolongation provides an easily accessible readout that might represent changes in voltage-dependent ion channels in APP695SWE mice, possibly under cholinergic control.

P2-01-16

Neuroprotection effect of Ginsenoside Rb1 and geniposide in rat cultured hippocampal neurons

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Ginsenoside Rb1 and geniposide have been extensively used in traditional oriental medicine for the prevention and treatment of ischemic stroke in patients. Recent studies showed that ischemic stroke patient might be more likely to experience memory decline and increased the risk of Alzheimer's disease. Herein, we observed whether Rb1 and geniposide could be a therapeutic agent for

Alzheimer's disease. Accumulating evidence suggests that ginsenoside Rb1 promotes neurotransmitter release through the PKA pathway. Geniposide shows a greater protective effect via MAPK signaling pathway in PC12 cells. Our *in vitro* studies using rat cultured hippocampal neurons revealed that the compounds can prevent the toxicity of Alzheimer's amyloid beta protein (Aβ). The cultured hippocampal neurons had conspicuously degenerated treatment with $A\beta_{25\text{-}35}$, but the compounds can reduce the neuronal death by morphological observations and MTT assay. These data suggested the possible mechanism of the protective effect of compounds against $A\beta_{25\text{-}35}$ induced cytotoxicity. Moreover, the compounds can enhance the growth of rat hippocampal neurons. Immunocytochemical staining and western blotting indicated that the treated neurons expressed more neuron-specific neurofilament-L than the non-treated. We calculated the length of neurites per cultured hippocampal neuron and this implied that the compounds can regulate some neuronal functions such as neurite extension and dendrite maturation. In conclusion, Rg1 and geniposide inhibit the neurons death against $A\beta_{25\text{-}35}$ induced cytotoxicity and increase the survival chance of the primary cultured neurons. Also, the compounds can preferentially direct neurite extension so promote neurite growth. A newly identified active ingredient of drug might be a novel preventive candidate in treating neurodegenerative disorders.

P2-01-17

Aβ generation inhibition of curcumin is associated with regulation of GSK-3β and amyloid precursor protein

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Previous reports suggest that both GSK-3β and amyloid precursor protein (APP) play important roles in pathogenesis of Alzheimer's disease (AD). Curcumin (diferuloylmethane) has been shown to have therapeutic properties on Alzheimer's disease, inhibit the generation of Aβ, but the mechanism is not fully defined. Plasmids APPswe and BACE1-mychis were transiently co-transfected in SH-SY5Y and HEK293 cells by LipofectaminTM 2000. The two cell lines then treated with curcumin to elude dose and time-dependent data. Quantitative RT-PCR was performed to measure GSK-3β and APP mRNA and Western Blot to detect the protein expression of GSK-3β and APP C99, the major β- secretase cleavage product. The concentration of Aβ40/42 was detected by ELISA. Results showed that the mRNA levels of GSK-3β and APP were decreased in a dose- and time-dependent manner ($p < 0.05$). Furthermore, this change was more significant in transfected SH-SY5Y cells. Western Blot showed that the expressions owere decreased in a dose- and time-dependent manner ($p < 0.05$). ELISA results showed that the generation of Aβ40/42 reduced significantly, also in a dose and time-dependent manner ($p < 0.05$). Our findings demonstrated that curcumin could inhibit the expression of GSK-3β and APP at mRNA and protein levels, and there is a linear relation between the decreased levels of GSK-3β expression and APP cleavage after treatment with curcumin. Reduced activation of GSK-3β was associated with decreased levels of APP resulted in decreased Aβ generation. Our study indicates that Aβ40/42 generation inhibition effect of curcumin might be due to its influence on the regulation of GSK-3β and APP.

P2-01-18

Peripheral neprilysin reduces brain amyloid in a transgenic mouse model of Alzheimer's disease

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Neprilysin (NEP) is a zinc metallopeptidase that efficiently degrades the amyloid beta peptides (Aβ) believed to be the causative agent of Alzheimer's disease (AD). To develop a new and tractable therapeutic approach for treating AD using NEP gene therapy, we constructed an adeno-associated virus (AAV) vector carrying the mouse NEP gene and injected various amounts of AAV into the hind limb muscle tissue of 3 × Tg-AD mice, a transgenic mouse model of Alzheimer's disease. We show that a sustained level of NEP is expressed for 6 months in muscle tissue companying a significant decrease of brain Aβ. Our findings demonstrate that gene therapy using NEP or other peptidases mediated by AAV is a promising application for AD. This is the first report of successfully using an Aβ degrading?peptidase? expression in peripheral muscle for treating AD.

P2-01-19

Characterisation of PINK1 knockout mice

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Parkinson's disease (PD) is the second most common neurodegenerative disease. It affects ~ 1% of the population above the age of 60. The classical form of the disease is characterised clinically by rigidity, resting tremor and bradykinesia. The pathological hallmarks of PD are loss of dopaminergic neurons in the substantia nigra and the formation of intracytoplasmic inclusion bodies (Lewy bodies). Although most PD cases are sporadic, mutations in some genes are known to cause rare familial forms of PD and the discovery of those mutations has shed light on our understanding of the molecular mechanisms contributing to the sporadic cases. Recently, several mutations in the one of these genes, namely PINK1, are found associated with autosomal recessive PD cases. PINK1 is reported to be a mitochondrial protein and has a highly conserved protein kinase domain with putative serine/threonine kinase activity. Functional *in vitro* studies strongly suggest a neuroprotective role for PINK1 in mitochondrial dysfunction and PD. In our study, we investigated the role of PINK1 in mitochondrial dysfunction and PD through multidisciplinary analysis of PINK1 knockout mice. Mitochondrial morphology and function has been examined by histopathology, ultrastructural, and biochemical analysis. Furthermore, aged PINK1 knockout mice were analysed for pathological hallmarks of PD, which including neuronal loss and aggregate formation.

P2-01-20

Mapt related gene network analysis for Parkinson's disease

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Parkinson's disease (PD) is a progressive, late-onset neurodegenerative disorder. Previous studies have identified microtubule-associated protein tau gene, *Mapt*, as a key gene of PD in human. In this study, we have combined array analysis and QTL mapping approach (genetical genomics) to characterize the genetic variation and regulatory network of *Mapt* in mouse. We identified that *Mapt* transcript in hippocampus is cis-regulated with likelihood-ratio statistic (LRS) 70 in BXD recombinant inbred (RI) strains (n = 67). Polymorphism analysis of *Mapt* revealed that eight SNPs between two BXD parental strains – C57BL/6J and DBA/2J in the promoter, which has a marked effect on steady-state levels of *Mapt* mRNA. Whole-brain *in situ* hybridization analysis (Allen Brain Atlas) showed that *Mapt* is well expressed in hippocampus. In addition, network analysis demonstrated that *Mapt* co-varies with many PD-related genes, including *Lrrk2* and *Pink1*. We also characterized numerous novel genes in the network that may be related to PD. However, their biological roles for PD require further investigation. The genetical genomics approach is proved extremely useful for identifying genes and pathways that contribute to neurodegenerative disease.

P2-01-21

C-Terminal truncation and Parkinson's disease-associated mutations down-regulate the protein serine/threonine kinase activity of PINK1

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The Parkinson's disease (PD) causative *PINK1* gene encodes a mitochondrial protein kinase called PTEN-induced kinase 1 (PINK1). The autosomal recessive pattern of inheritance of *PINK1* mutations suggests that PINK1 is neuroprotective and therefore loss of PINK1 function causes PD. Indeed, depletion of PINK1 renders neurons susceptible to neurotoxin-induced apoptosis. As a protein kinase, PINK1 presumably exerts its neuroprotective effect by phosphorylating specific cellular proteins and in turn modulating their functions. Towards elucidation of the neuroprotective mechanism of PINK1, we expressed the recombinant protein consisting of the PINK1 kinase domain either alone (PINK1 [KD]) or with the PINK1 C-terminal tail (PINK1 [KD+T]). Both recombinant enzymes preferentially phosphorylate the artificial substrate histone H1 at serine and threonine residues, demonstrating that PINK1 is a protein serine/threonine kinase. Introduction of the PD-associated mutations, G386A and G409V significantly reduces PINK1 [KD] kinase activity. Since Gly-386 and Gly-409 reside in the conserved activation segment of the kinase domain, the results suggest that the activation segment is a regulatory switch governing PINK1 kinase activity. PINK1 [KD+T] is more active than PINK1 [KD]. Both PINK1 enzymes exhibit different selectivity towards phosphorylation sites in histone H1, suggesting that the C-terminal tail contains determinants governing PINK1 kinase activity and substrate selectivity. Results of future investigation to identify physiological protein substrates of PINK1 with PINK1 [KD+T] will shed light on the mechanism of pathogenesis of PD.

P2-01-22

The initial study of brain proteomics of senescence-accelerated mouse

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Alzheimer's disease (AD) is one of the most common and complex neurodegeneration disorder characterized by senile plaques, neurofibrillary tangles and synapse loss. The senescence-accelerated prone mouse strain 8 (SAMP8) is an accelerated aging model and exhibits some similarities to AD. Here, it was used to investigate the pathogenic mechanisms involved in age-related learning and memory deficits which is relevant to aging and AD. The total proteins of hippocampus and cortex were extracted from 5 month-old, 10 month-old and 15 month-old SAMP8 mice and age-matched SAMR1 (control) mice. 2-D electrophoresis and MALDI-TOF-TOF-MS were performed to compare and identify proteins that were expressed differentially between the three age grades. The results showed that about 1,700 proteins were separated by each 2-DE gel. Compared with the same age SAMR1, Ubiquitin carboxy-terminal hydrolase-L3 was decreased, and mitochondrial inner membrane protein and adenylate kinase 4 were increased significantly in the hippocampus and cortex of all the three age grades. EF hand domain containing 2 molecular weight slightly decreased in the hippocampus of 10, 15 month-old SAMP8 mice and the cortex of the three age grades SAMP8 mice, and heme binding protein 1 was decreased only in the hippocampus of 15 month-old SAMP8. In conclusion, there were significant differences in hippocampus and cortex protein expressions between SAMP8 and SAMR1 which were related to mitochondrion function, energy metabolism, neuroprotectin, signal transduction. These proteins would provide clues for the study of pathological mechanisms of AD and might be the new possible drug targets for AD therapy.

P2-01-23

PINK1 is necessary for long term survival and mitochondrial function in primary human dopaminergic neurons

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Parkinson's disease (PD) is a common age-related neurodegenerative disease and it is critical to develop models which recapitulate

the pathogenic process including the effect of the ageing process. Although the pathogenesis of sporadic PD is unknown, the identification of the mendelian genetic factor PINK1 has provided new mechanistic insights. In order to investigate the role of PINK1 in Parkinson's disease, we studied PINK1 loss of function in human and primary mouse neurons. Using RNAi, we created stable PINK1 knockdown in human dopaminergic neurons differentiated from foetal ventral mesencephalon stem cells, as well as in an immortalised human neuroblastoma cell line. We sought to validate our findings in primary neurons derived from a transgenic PINK1 knockout mouse. For the first time we demonstrate an age dependent neurodegenerative phenotype in human and mouse neurons. PINK1 deficiency leads to reduced long-term viability in human neurons, which die via the mitochondrial apoptosis pathway. Human neurons lacking PINK1 demonstrate features of marked oxidative stress with widespread mitochondrial dysfunction and abnormal mitochondrial morphology. We report that PINK1 plays a neuroprotective role in the mitochondria of mammalian neurons, especially against stress such as staurosporine. In addition we provide evidence that cellular compensatory mechanisms such as mitochondrial biogenesis and upregulation of lysosomal degradation pathways occur in PINK1 deficiency. The phenotypic effects of PINK1 loss-of-function described here in mammalin neurons provide mechanistic insight into the age-related degeneration of nigral dopaminergic neurons seen in PD.

P2-01-24

Effect of global ischemia and advanced age on metabolism of amyloid precursor protein

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Manifold processes can contribute to cell death in the brain. Current data suggest that Alzheimer's disease is linked to ischemia-reperfusion brain injury. In the present study we investigated effects of global 15 min ischemia (the four-vessel occlusion model) and 120 min reperfusion on the level of amyloid precursor protein (APP) and some amyloid peptide (Aβ) degrading metalloproteinases in adult and old (15 months) male rats. We have found that ischemia results in a significant increase of APP levels. Changes in APP levels were more dramatic in adult animals. Levels of APP were significantly increased after ischemia compared to reperfusion. On the contrary, levels of endothelin-converting enzyme (ECE) were decreased significantly after ischemia in adult animals. After reperfusion ECE levels were significantly increased compared to control as well as to adult animals after ischemia. The amount of ECE did not change after ischemia insult in old animals. Levels of β-secretase (BACE1) - a key enzyme in Aβ plaque formation in AD, were significantly increased after ischemia in adult animals but not in old animals. After ischemia we have also observed oxidative damage of proteins. The levels of dityrosine and lysine conjugates with products of lipid peroxidation were increased after ischemia. Levels of free sulfhydryl groups and thiobarbituric acid-reactive substances did not change after ischemia in adult animals. The amount of thiobarbituric acid-reactive substances increased after ischemia in old animals. Our results suggest that ischemia may lead to accumulation of amyloid peptide and can induce reactive oxygen species formation, protein oxidation and lipid peroxidation in neurons.

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P2-01-25

The effect of gastrodin on the expression of TNF-α and GDNF in the rat of Parkinson's disease

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Parkinson's disease (PD) is characterized by a progressive loss of dopamine (DA) containing neurons in the substantia nigra (SN). The symptoms of the disease occur when > 80% of the DA content in the striatum is lost due to this degeneration. Current PD research focuses on the investigation of drugs combining antiparkinsonian and neuroprotective effects. Gastrodin is known to have antiparkinsonian activity and to reduce side effects of levodopa treatment. Both *in vitro* and *in vivo* experimental studies suggest this drug may also have neuroprotective properties. Therefore, we investigated the possible neuroprotective effect of gastrodin to reveal the effect of different dose of gastrodin on the mechanism of neuroinflammation in the rat of Parkinson's disease. Rats received a unilateral 6-hydroxydopamine (6-OHDA) injection into the left striatum. Testing was carried out 2 weeks post-lesioning after gastrodin intervention. The expression of tumor necrosis factor-α (TNF-α) and glial cell line-derived neurotrophic growth factor (GDNF) was evaluated by TNF-α and GDNF staining. Small doses of gastrodin treatment, significantly attenuated the expression of TNF-α and up GDNF. In 6-OHDA lesion striatum model, for PD, small dose of gastrodin treatment not only significant attenuated the expression of TNF-α, but also up the expression of GDNF. Hence, gastrodin may adjust the mechanism of neuroinflammation in Parkinson's disease.

P2-02-01

Role of ATP in memory formation

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The nucleotide ATP is recognized as a neurotransmitter is involved in neural-glial interactions in the brain and has been suggested to have a pivotal role in learning and memory. ATP is released from both neurones and astrocytes and activates ionotropic (P2X) or metabotropic (P2Y) purine receptors found on neurones, astrocytes and capillaries in the CNS. All three activate different purinergic receptors with distinct pharmacology. A single, brief learning experience with a bitter tasting bead is used. A weak version where memory lasts for only 30 min, presents the opportunity to enhance memory consolidation and facilitate the formation of long-term memory. We have recently found that hippocampal injections of ATP promote the formation of memory after weakly-reinforced learning. To understand which of the purinergic receptor subtypes are involved in memory processing, we have injected selective

purinergic agonists and antagonists into the hippocampus and examined their effects on memory. Specific purinergic P2Y receptors are involved in memory consolidation formation at two critical times: 0–2.5 min and at 30–35 min after training. ATP-γ-S, ADP-β-S, and UTP all enhanced weak learning. ATP is a co-transmitter often released with glutamate, and in the chick hippocampus, glutamate release occurs at these two same time periods. The importance of the latter period is that labile memory is consolidated into permanent memory. The earlier time relates to acquisition and short-term memory storage. We have also shown that inhibition of NMDA and AMPA receptors in the chick hippocampus can interfere with memory storage at these times.

P2-02-02

Chromatin factors in the regulation of MAP2 gene during atRA induced differention of embryonal carcinoma cells

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Chromatin remodeling, an epigenetic phenomenon, provides an open conformation of chromatin to facilitate eukaryotic gene transcription. The local changes are necessary for transcriptional regulation. Microtubule-associated protein 2 (MAP2) is a major component of the cross-bridges and is implicated in the cytoskeletal protein abnormalities in neurodegenerative diseases, Embryonal Carcinoma Cells (P19) treated with *All Trans* retinoic acid *(*atRA) was used as a model. A dramatic increase of MAP2 was found at both of mRNA and protein levels in the atRA treated P19 cells determined by qPCR, Western blot and immunofluorescence assays. However, a significantly reduction of MAP2 mRNA was observed when trichostatin A was added with atRA treatment. We also showed in atRA treated p19 cells that the promoter activity of a 1.3kb MAP2 construct was 5-6 folds higher than without atRA treatment. When TSA was co-treated the cells with atRA, it was also reduced. Furthermore, expression plasmids of Brm and Brg1 ATPases of the SWI/SNF chromatin remodeling complexes, pCAF and p300 of the histone acetyltransferases were individually cotransfected with the MAP2-1.3k-luc. It was found in atRA treated cells that Brg1 and pCAF enhanced the promoter activity for about 5 and 4 folds, respectively. Brg1 interaction with the proximal flanking sequence of MAP2 promoter was detected by ChIP. However, Brm and p300 showed no obvious effect. These data suggested that the transcription of MAP2 could be regulated at the chromatin level either via the ATP dependent chromatin remodeling or histone acetylation and shed lights on target for the prevention or therapy of neurodegenerative diseases.

P2-02-03

Learning and memory impairment in lipoprotein lipase deficient mice is caused by presynaptic defects

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Lipoprotein lipase (LPL) is predominantly expressed in adipose, muscle and macrophages and plays a crucial role in the metabolism of triglyceride-rich plasma lipoproteins. In the brain LPL is most highly expressed in the pyramidal cells of the hippocampus suggesting that LPL may be involved in cognitive function. However, very little is known about the role of LPL in the brain. In order to elucidate the role of LPL in brain function we have used a mouse model of LPL deficiency. Previously, we rescued otherwise lethal neonatal mice deficient in LPL gene by somatic gene transfer resulting in some amelioration of the lipid phenotype but no LPL expression in the brain. To assess learning and memory function in these mice, we tested their performance in a water maze test and the step-down inhibitory avoidance task. LPL deficient mice exhibited impaired performance in the water maze task including increased latency to escape platform and increased mistake frequency compared to wild-type mice. Increased latency to platform in the step-down test was also observed. As transmission electron microscopy experiments revealed a significant decrease in the number of pre-synaptic vesicles in the hippocampus of LPL deficient mice, the levels of pre-synaptic marker synaptophysin were reduced in the hippocampus of LPL deficient mice. No changes in the levels of the post-synaptic marker PSD-95 were observed. Theses findings indicate that LPL plays an important role in learning and memory function possibly by affecting pre-synaptic processes in the hippocampus.

P2-02-04

Presynaptic defects impair learning and memory function in lipoprotein lipase deficient mice

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Lipoprotein lipase (LPL) is a crucial role in the metabolism of triglyceride-rich plasma lipoproteins and predominantly expressed in adipose, muscle and macrophages. In the brain LPL is most highly expressed in the pyramidal cells of the hippocampus suggesting that LPL may be involved in cognitive function. In order to elucidate the role of LPL in brain function we have used a mouse model of LPL deficiency. Previously, we rescued otherwise lethal neonatal mice deficient in LPL gene by somatic gene transfer resulting in some amelioration of the lipid phenotype but no LPL expression in the brain. A water maze test and the step-down inhibitory avoidance task were performed to assess learning and memory function in these mice. LPL deficient mice exhibited impaired performance in the water maze task including increased latency to escape platform and increased mistake frequency compared to wild-type mice. Increased latency to platform in the step-down test was also observed. Apoptosis in the hippocampus was not appreciable with DNA extraction and agarose gel electrophoresis and TUNEL assay. However, as transmission electron

microscopy revealed a significant decrease in the number of pre-synaptic vesicles, the level of pre-synaptic marker synaptophysin was reduced in the hippocampus of LPL deficient mice. No changes in the protein level of the post-synaptic marker PSD-95 and PSD length were observed. Theses finding indicate that LPL plays an important role in learning and memory function possibly by affecting pre-synaptic processes in the hippocampus.

P2-02-05

Regional variations of agmatine and putrescine levels in memory-associated brain structures

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The hippocampus and its adjacent entorhinal, perirhinal and parahippocampal (postrhinal in rodents) cortices, as well as the prefrontal cortex, play important roles in certain types of learning and memory. It has been shown that declined learning and memory ability in aged individuals is associated with the dysfunction of these memory-related structures. Agmatine, a metabolite of L-arginine, is a novel neurotransmitter and increasing evidence suggests its neuroprotective effects and modulatory role in learning and memory. Putrescine, a positively charged aliphatic amine, modulates learning and memoey and plays an important role in hippocampal neurogenesis, and DNA, RNA and protein synthesis. The present study measured agmatine and putrescine levels in the memory-associated brain structures in 4-month-old male Sprague Dawley rats using liquid chromatography/mass spectrometry. For agmatine, the highest level was found in the prefrontal cortex followed by the perirhinal cortex, hippocampal dentate gyrus (DG) and CA1, the postrhinal cortex, the CA2/3 region of the hippocampus and the entorhinal cortex. For putrescine, the CA1, CA2/3 and DG sub-regions of the hippocampus had much higher levels as compared to the cortical regions examined. These findings, for the first time, demonstrate the regional variations of agmatine and putrescine in memory-associated brain structures. Future research will focus on their contributions to age-related deficits in learning and memory and hippocampal neurogenesis.

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P2-02-06

Aging alters agmatine levels in memory-associated brain structures

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Aging is a multifactorial process and leads to cognitive decline. Agmatine is a metabolite of L-arginine by arginine decarboxylase. Recent evidence suggests that it exists in mammalian brain and is a novel neurotransmitter. The present study measured agmatine levels in several memory-associated brain structures in aged (24-month-old), middle-aged (12-month-old) and young (4-month-old) male Sprague Dawley rats using liquid chromatography/mass spectrometry. Agmatine levels were significantly decreased in the CA1, but increased in the CA2/3 and dentate gyrus, sub-regions of the hippocampus in aged and middle-aged rats relative to the young adults. In the prefrontal cortex, a dramatic decrease in agmatine level was found in aged rats as compared to middle-aged and young rats. There were significantly increased levels of agmatine in the entorhinal and perirhinal cortices in aged relative to middle-aged and young rats. In the postrhinal and temporal cortices, agmatine levels were significantly increased in aged and middle-aged rats as compared to young adults. The present findings, for the first time, demonstrate age-related changes in agmatine levels in memory-associated brain structures and raise a novel issue of the potential involvement of agmatine in the aging process.

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P2-02-07

Different expression of PDGFRα in the different brain regions of the rat at different postnatal time

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The objective is to study expression of Platelet-derived growth factor receptorα (PDGFRα) in the different brain regions of the rat at different postnatal time and explore PDGFRα function in nervous system development. Respectively extract mRNA and protein from cerebra, hippocampus and cerebellum on postnatal 7 days (P7), 14 days (P14) and 28 days (P28). PDGFRα mRNA expression level was detected using Real-time PCR and PDGFRα protein level was analyzed using Western Blot. In cerebra, PDGFRα mRNA level on P14 is higher than P7 and P28, but PDGFRαprotein increased gradually from P7 to P14 (the expression level on P28 is 2 times higher than that on P7). In hippocampus, PDGFR αmRNA expression level keeps steady relatively from P7 to P28 and the change of protein expression is similar to that of mRNA. But in cerebellum PDGFRα mRNA and protein expression all decreased gradually from P7 to P28 (PDGFRα protein level on P7 was 5 times high of its on P28). In conclusion, PDGFRα maybe have an important role in regulating postnatal brain development.

P2-02-08

Human stem cells to assess the developmental neurotoxicity: monochrotophos induced changes in marker genes

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Attempts were made to study the influence of monochrotophos (MCP), known neurotoxic organophosphate pesticide, on the expression of neural specific marker genes in chemical induced differentiating neural cells derived from human umbilical cord blood stem cells (hUCBSC). Cells were induced to differentiate into neural subtypes in neurobasla medium supplemented with B-27, N-2, NGF, dibutile butyryl cAMP, IBMX, BDNF, bFGF, retinoic

acid, and TPO. Non-cytotoxic doses of MCP, exerting effect on growth and differentiations were ascertained in differentiating cells at day 0, 6, 12, 18, 24 and 30. For expression studies, undifferentiated cells were exposed to selected concentrations of MCP. Following MCP exposure, cells were allowed to differentiate to neuronal subtypes. MCP induced alterations in neural specific marker genes viz., nestin, beta-tubulin III, MAP-2, TH, NF-M, NeuN, DA-D_2 and GABA were studied. Significant expression of nestin and beta-tubulin III could be detected on day 6 of differentiation, whereas most of the cells were expressing mature neural markers and displayed typical neural cell morphology with developed dendrites by day 18. However, increase in the magnitude of these neural markers was insignificant except morphological differentiation. MCP (10^{-5}M) exposure for 60 min onwards could cause significant ($p < 0.001$) alterations in the expression of early and mature neural makers genes. Interestingly, early stages were found to be more vulnerable to MCP (10^{-5} M) then the cells at late maturation. No significant alteration could be recorded following MCP (10^{-6}M). Data indicates the potential application of differentiating neural cells derived from hUCBSC to understand the mechanisms involved in developmental neurotoxicity.

P2-02-09

Heroin exposure in uterus elicit differential effects on MAP Kinase (JNK, ERK and p38) signaling pathways in PFC and hippocampus

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More and more heroin abusers were found to be women of bearing-age any offspring is in high risk of neurobehavioral teratogenicity and vulnerability of neural degeneration disease. Although the behavioral defect has been widely reported the signaling mechanisms that link mitogen-activated protein kinase (MAPK) pathway to destructive cellular responses to heroin-exposure in uterus are poorly understood. We aim to investigate the involvement of MAPK signal pathways. MAPK family compose a family of extracellular signal-regulated kinase (ERK), p38, and c-Jun N-terminal kinase (JNK) and are important mediators of signal transduction that play a key role in the regulation of many essential cellular processes, such as cell growth, proliferation, differentiation, and apoptosis. This study examined the activation of JNK, ERK, and p38 MAPK in response to prenatal heroin exposure. Animal model was established by administrating Diacetylmorphine to pregnant BALB/c mice on the embryonic days 8-19. The offspring were divided into 2 groups heroin-treated (HER) group, received 10 mg/kg heroin subcutaneously, from E8 to E19 daily and saline (SAL) groups. Morphological methods; immunohistocytochemistry, RT-PCR and Western Blot were employed to evaluate the MAPK expressions on mRNA level and protein level of phosphor-ERK (p-ERK), p-p38 and p-JNK MAPK in prefrontal lobe cortex and hippocampus. Data of three methods showed coherent alteration of three MAPK members: significant activation of p-p38 and p-JNK and concomitant inactivation of p-ERK MAPK activity. Prenatal heroin exposure can elicit distinct and specific effects on MAPK-mediated signaling pathways, the alterations of P38 and JNK MAPK may participate in the heroin neurobehavioral teratogenicity.

P2-02-10

Neurogenin1 is regulated by Sox6 in RA induced neuronal differentiation of P19 embryonic carcinoma cells

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Basic helix-loop-helix (bHLH) proteins are expressed in most stages in neural lineages during development and play crucial role in the determination of cell fates. Neurogenin1 (Ngn1) is an important member of bHLH proteins, which can induce neurogenesis and prohibit the differentiation of neural stem cells into astrocytes. Sox6 is a member of SRY-related high-mobility-group box (Sox) proteins, and plays an important role in the development of the central nervous system (CNS). However, the mechanism of the regulation of Sox6 in the development of the CNS is still unclear. In our study, we used P19 embryonic carcinoma cells treated with retinoic acid (RA) as a model for studying neuronal differentiation and found that ngn1 was regulated by Sox6 during the neuronal differentiation of P19 cells. In RA-induced differentiation of P19 cells, the expression of ngn1 markedly increased. With bioinformatic method, several predicted binding sites of Sox proteins were found at the promoter of ngn1. We showed that Sox6 could bind to the upstream HMG consensus site of ngn1 promoter with EMSA assay. The expression of Sox6 increased when treated with RA for 2 days, and then decreased during the neuronal differentiation. Our results suggested that Sox6 could directly bind to the promoter and activate the expression of ngn1. These findings provide a novel insight into the function of Sox6 in neuronal differentiation and are helpful for us to understand the details of CNS development.

P2-02-11

DIXDC1 promotes retinoic acid induced neuronal differentiation and inhibits gliogenesis in P19 cells

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Human DIXDC1 is a member of Dishevelled-Axin (DIX) domain containing gene family which plays important roles in Wnt signaling and neural development. In this report, we first confirmed that expression of Ccd1, a mouse homologous gene of DIXDC1, was upregulated in embryonic developing nervous system. Further studies showed that Ccd1 was expressed specifically in neurons and colocalized with early neuronal marker Tuj1. During the aggregation induced by RA and neuronal differentiation of embryonic carcinoma P19 cells, expressions of Ccd1 as well as Wnt-1 and N-cadherin were dramatically increased. Stable overexpression of DIXDC1 in P19 cells promoted the neuronal differentiation. P19 cells overexpressing DIXDC1 but not the control P19 cells could differentiate into Tuj1 positive cells with RA induction for only 2 days. Meanwhile, we also found that overexpression of DIXDC1 inhibited gliogenesis in P19 cells by downregulating the expression

of GFAP. Thus, our finding suggested that DIXDC1 might play an important role during neurogenesis; overexpression of DIXDC1 in embryonic carcinoma P19 cells promoted neuronal differentiation and inhibited gliogenesis induced by retinoic acid.

P2-03-01

Role of 14-3-3 protein in neuronal death and differentiation

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The 14-3-3-protein family plays critical regulatory roles in signaling pathways in cell division and apoptosis. However the exact physiological functions of the 14-3-3 proteins in neurons remain unclear. To address this question, we studied the role of endogenous 14-3-3 proteins in neuroblastoma cells (N2a) by using difopein (dimeric fourteen-three-three peptide inhibitor), which specifically blocking the interaction of 14-3-3 and its ligands. Expression of difopein in N2a cells caused nearly 12% and 46% of total cell death in N2a cells after 24 and 48 h respectively by quantitative measurement. Hoechst staining showed that difopein-caused cell death was apoptosis. Western blotting analysis demonstrated that activated caspase-3 increased evidently 24 h after difopein transfection. Overexpression of difopein in primary culture of cerebral cortical neurons also caused severe cell death. These data indicated that 14-3-3 proteins were essential in the survival of neurons. Interestingly, we found that in addition to cell death, difopein overexpression induced a prominent neurite outgrowth in survived N2a cells. Akt was activated remarkably upon Difopein expression, suggesting that 14-3-3 regulates neuronal differentiation through PI-3/Akt pathway. Our data suggested that 14-3-3 plays pivotal roles in controlling both cell death and differentiation in neuronal cells.

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P2-03-02

The bipotential influence of GAP-43 on neuronal death, survival and regeneration

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The 43-kD growth-associated protein (GAP-43) in the nervous system has attracted much attention because its properties are associated with axonal growth during development and axonal regeneration in the adult. Certain reports have demonstrated however, that an increased expression of GAP-43 *in vivo* may induce unexpectedly cellular apoptosis during the development and regeneration in the nervous system, indicating that GAP-43 contributes not only to axonal growth but also involvement in neuronal cell death. Here, we put forward this hypothesis and hta it depended on some repulsive signals from extrinsic environment which may switch the function of GAP-43 from growth associated to death associated. We firstly obtained the GAP-43 overexpressing MN9D cells, utilizing retroviral-mediated gene transfer system. The cells with overexpression of GAP-43 showed to have a promotion of neurite growth and nerve sprouting, and no influence on the cell viability in a favorable condition. Increase of the cellular GAP-43 level, however, led to these cells susceptible to the conditional medium containing the repulsive guidance cue, sema3A. Under this condition medium, the number of death cells was increased significantly and this was caused by apoptosis, proved by TUNEL and other method. The different calpain inhibitors, including PD150606 and calpeptin could block this facilitation of Sema3A-induced apoptosis. The results in present study may support our hypothesis that the GAP-43 is a bipotential mediator for neuronal death, survival and regeneration, and the proteolysis at Ser41 by calpain may be involved in regulation of the functional conversion of this protein in the reaction to an extracellular inhibitory environment.

P2-03-03

Effects of NP7 on the cell death induced by oxidative stress in neuronal and glial midbrain cultures from parkin null mice

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Parkin mutations account for the most frequent and best characterized cause of Parkinson's disease (PD). PD is a multifactorial disease which has been often related to oxidative stress. Suppression of parkin in mice produces dopamine cell death attributed to abnormally high free radical production mediated by an enhanced intracellular metabolism of dopamine. The synthetic, marine-derived neuroprotectant NP7 is a free radical scavenger with good penetration into the brain which has shown neuroprotective effects in several *in vitro* models of neurodegenerative disease. We have examined the effect of NP7 on H_2O_2 mediated cell death in neuronal and glial cultures from WT and parkin null mice (PK-KO). We used fetal WT and PK-KO midbrain neuronal-enriched and glial cultures. Preliminary dose response experiments allowed us to identify the optimal dose of NP7 for our cultures in the range of 5–10 μM. We measured apoptosis by chromatin condensation with bis-benzimide, and necrosis by LDH and trypan blue assays. Mitochondrial activity was evaluated with MTT assay and the effects in the different cells population by immunocytochemistry. GSH and H_2O_2 levels were measured by spectrophotometry. We found that NP7 has a protective effect on both WT and PK-KO neuronal-enriched and glial cultures from the H_2O_2-induced toxicity. This effect is mediated through a reduction of the levels of H_2O_2 in the medium and a concomitant reduction of apoptosis and necrosis. NP7 protects DA neurons from the induced-cell death and abolished the microglial activation and is a compound potentially useful for the neuroprotective treatment of patients with Parkinson's disease.

P2-03-04

Apoptogenic mitochondrial signaling and protease activation in striatal GABAergic neurons: insult-dependent effects relevant to Huntington's disease

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Injury of striatal GABAergic neurons in Huntington's disease, involves excitotoxicity, metabolic and oxidative stress, which recruit apoptogenic mitochondrial signaling. This study aimed to elucidate the insult-dependent recruitment of caspase-dependent/-independent 'death' signaling in primary striatal cultures. Using E18 C57Bl6 mice, insults targeting various injury processes were analysed. At 6d drugs (3-nitropropionate (3-NP), 3-morpholinosyndnonimine (SIN-1), N-methyl-D-aspartic acid (NMDA), 3, 5-dihydroxyphenylglycine (DHPG) & staurosporine) were added (24 or 48h) in the presence and absence of inhibitors of caspase-9, caspase-3 and calpains. Insults produced concentration-dependent injury, which at EC50 concentrations were shown to be apoptotic by patterns of annexin V and propidium iodide (PI) labeling. Fast and slow timecourses of injury were delineated and employed. Double immunocytochemistry of the redistribution of cytochrome c, second mitochondrial activator of caspases (Smac), high temperature requiring protein A2 (HtrA2/Omi) and apoptosis-inducing factor (AIF), recruitment of caspase-dependent and -independent 'death' signaling, respectively, was quantitated. STS affected classical activation of the intrinsic mitochondrial pathway with early release cytochrome c and Smac, and late release of HtrA2/Omi and AIF. 3-NP and NMDA, unlike STS, produced earlier release of AIF and HtrA2/Omi, while SIN-1 displayed an intermediate release pattern, suggestive of differential profiles of caspase-independent injury. Protease inhibition failed to attenuate patterns of insult-induced PI-labeling indicating the redundancy of death pathways when cells were committed to die. NMDA and DHPG, produced prominent calpain cleavage product, SIN-1 and 3-NP both calpain and caspase products, whilst STS exhibited predominantly caspase activation. These results indicate apoptosis of striatal GABAergic neurones by pathologic insults involves substantial calpain activation and caspase-independent AIF redistribution.

P2-03-05

Lysophosphatidic acid-induced survival of immortalized hippocampal progenitor cells by inactivation of glycogen synthase kinase 3 in pertussis toxin-sensitive pathways

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Lysophosphatidic acid (LPA) is a lipid growth factor shown to regulate cell proliferation, survival, and death. Tight regulation of neuronal precursor cell survival is essential during neurogenesis in both developing and adult brain. Increasing study have shown that various extracellular factors including LPA plays roles in controlling cell survival and apoptosis in early developing neurons. However, detailed control mechanisms underlying LPA-induced neuronal survival remain unclear. To explore how LPA regulates cell survival or apoptosis in developing neuron, signaling cascades triggered by LPA in neuronal survival were investigated in H19-7 cells. Here, we report that LPA promoted cell survival by suppressing apoptosis. LPA stimulated serine phosphorylation of glycogen synthase 3 (GSK-3) α/β. And inactivation of GSK-3β by PMA and BIO, GSK-3β specific inhibitor, also prevent H19-7 cells apoptosis. GSK-3β phosphorylation along with LPA-induced survival was suppressed by pertussis toxin (PTX) and by siRNA for LPA1 or LPA2. Taken together, these results demonstrated that LPA induced-cell survival occurs through $G_{i/o}$ coupling of the LPA receptors following inactivation of GSK-3 in H19-7 cells.

P2-03-06

Deficiency in endothelin receptor B reduces proliferation of neuronal progenitors and increases apoptosis in postnatal rat cerebellum

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Endothelins regulate cellular functions in the mammalian brain through the endothelin receptors A and B (EDNRA and EDNRB). In this study, we investigated the role of EDNRB on cell proliferation in the cerebellum by using the spotting lethal (sl) rat, which carries a naturally occurring deletion in the EDNRB gene. Proliferating cells in the three genotypes, wild-type (+/+), heterozygous (+/*sl*) and homozygous mutant (*sl/sl*) rats were labelled by intraperitoneal injection of 5-bromo-2'-deoxyuridine (BrdU) at postnatal day 2. The density of BrdU-positive cells (per mm^2) in the external germinal layer of *sl/sl* rats (Mean±SEM, 977 ± 388) was significantly reduced compared to +/+ (4915 ± 631) and +/*sl* (2304 ± 557) rats. Subsequently, we examined the effects of EDNRB mutation on neural apoptosis by terminal deoxynucleotidyltransferase-mediated dUTP nick end-labeling assay. This showed that the density of apoptotic cells in the cerebella of *sl/sl* rats (9.3 ± 0.5) was significantly increased than +/+ rats (4 ± 0.7). The expression of brain derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF) were measured with standard ELISA, but were unchanged in all genotypes. These results suggest that ENDRB mediates neural proliferation and have anti-apoptotic effects in the cerebellum of the postnatal rat, and that these effects are independent of changes in the expression of BDNF and GDNF. Our findings will lead to better understanding of the morphological changes in the cerebellum of Hirschsprung's disease patients with congenital EDNRB mutation.

P2-03-07

Neuroprotective efficacy of estrogen in acute and chronic spinal cord injury in rats

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Spinal cord injury (SCI) leads to neurological dysfunction and paralysis. Methylprednisolone renders limited protection in SCI. We

explored the neuroprotective efficacy of estrogen in SCI. Standard methods to induce acute and chronic SCI in rats were used. Acute SCI rats were treated with three doses of estrogen (10, 100 and 200 μg/kg) while chronic SCI rats were treated with only a low dose (10 μg/kg) and a high dose (4 mg/kg) of estrogen. Western blotting was used to monitor changes in Bax: Bcl-2 ratio and also the expression and activity of the proteolytic enzymes (e.g., calpain, caspase-3) and angiogenesis factors in SCI tissues. Moreover, we used SCI sections to perform single and double immunofluorescent stainings to examine proinflammatory responses such as astrogliosis and microgliosis, microvessel growth, expression of VEGF and angiogenic receptors and to identify TUNEL-positive neurons. Motor function was assessed in chronic SCI rats via BBB scale. Sham rats manifested negligible pro-inflammatory responses or proteolytic events and showed very low levels of neuronal death. In contrast, the vehicle-treated SCI rats showed profound proinflammatory responses with astrogliosis and microgliosis; elevated Bax:Bcl-2 ratio, enhanced expression and activity of calpain and caspase-3; reduced expression of angiogenic factors, and high levels of neuronal death in the lesion and caudal region of the spinal cord in acute SCI rats. Estrogen treatment not only reduced the proinflammatory and proteolytic activities in acute SCI, but also provided long lasting neuroprotection to chronic SCI. These results suggest estrogen as a promising therapeutic agent for treating both acute and chronic SCI.

P2-03-08

EGCG protect SH-SY5Y cells against 6-OHDA induced cell death through STAT3 activation

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EGCG has multiple neuroprotective actions such as anti-oxidant, anti-inflammation, iron-chelating in treating neurodegenerative diseases including Parkinson's disease and Alzheimer's disease. Recent studies have demonstrated that signal pathways also play an important role in the neuroprotective action of EGCG in Parkinson's disease. The purpose of this study was to investigate potential neuroprotective action of EGCG and the role of STAT signal pathway in the protective mechanisms of EGCG in order to prove whether STAT3 /STAT1 can act as a new diagnosis and treatment target of EGCG for PD. In this study, 6-OHDA treated human neuroblastoma (NB) SH-SY5Y cells were used as the cell model of Parkinson's disease. 6-OHDA (50-400umol/L) concentration dependently decreased the cell viability and pretreatment with EGCG (0.1-10umol/L) attenuated the cell death induced by a 24-h exposure to 6-OHDA (100umol/L). Whether STAT was involved in this neuroprotective effect was further examined. Western blotting show that 6-OHDA inhibited the activity of STAT3 in SH-SY5Y cells but pretreatment of EGCG for 15 min effectively restored the reduced STAT3 phosphorylation. These results suggest that disruption of STAT3 signaling by mediators of oxidative stress contributes to the neuronal damage observed in neurodegenerative diseases and STAT3 stimulation was involved in the neuroprotective mechanism of EGCG against oxidative stress induced cell death, EGCG can be used as therapeutic agents in preventing nerve cell death.

P2-03-09

Cdk1 phosphorylation and activation of FOXO1 promotes cell death in postmitotic neurons

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Activation of the mitotic kinase Cdk1 has been linked to cell death of postmitotic neurons in brain development and neurodegenerative diseases. However, the mechanisms by which Cdk1 triggers neuronal death and degeneration remain poorly understood. Here, we demonstrate that Cdk1 phosphorylates the transcription factor FOXO1 within the forkhead domain at Serine 249 *in vitro* and *in vivo*, and thereby induces cell death in primary neurons. Remarkably, in contrast to a report suggesting that Cdk2-induced Serine 249-phopshorylated FOXO1 is sequestered and inhibited in the cytoplasm, we found that the Cdk1- or Cdk2-induced Serine 249 phosphorylation stimulated FOXO1-dependent transcription in distinct cell types including primary neurons. The Cdk-induced phosphorylation of FOXO1 at Serine 249 disrupted FOXO1 binding with 14-3-3 proteins, providing a mechanism for the nuclear translocation and activation of FOXO1. These results define a signaling link between Cdk1-related kinases and FOXO1 that may play a key role in diverse biological processes including cell death and degeneration of postmitotic neurons.

P2-04-01

Pain hypersensitivity and neuropathy in insulin-resistant rats induced by high-fat-sucrose diets or high-fat-sucrose-salt diets

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Diabetic sensorimotor polyneuropathy (DSP) is one of common and severe diabetic complications which results in high mutilation and fatality rate. We examined whether sensorimotor functions are changed in rats with insulin resistance (pre-diabetes) induced by high-fat-sucrose diets (HFSD) and high-fat-sucrose-salt diets (HFSSD). .Insulin resistance was established in adult male Sprague-Dawley albino rats by long-term HFSD or HFSSD feeding. Rats fed conventional diets (CD) served as control. During the feed period (0-120 days), systolic blood pressure, sensorimotor functions, fasting blood glucose (FBG), plasma concentration of total free fatty acid (FFA) and insulin, homeostasis model assessment insulin resistance (HOMA-IR) index were measured. Finally, structures of large myelinated fibers (LMF), small myelinated fibers (SMF) and unmyelinated fibers (UMF) were observed and measured under EM, respectively. Rats fed both HFSD and HFSSD showed a significant increase in blood insulin or total FFA since 15 or 20 days, however, FBG remained as normal until termination of the experiment. HOMA-IR index indicated occurrence of IR in these rats. Parallel examination of pain sensitivity suggested occurrence of mechanical, but not thermal, hyperalgesia or allodynia in the IR rats. Motor coordination was also impaired in the IR rats. EM observations suggest an important contribution of LMF demyelination and

degeneration to the development of mechanical hyperalgesia in diet induced IR rats. Sensorimotor dysfunctions such as mechanical pain hypersensitivity and reduced motor coordination do occur earlier in HFSD or HFSSD-induced insulin resistant rats and selective disruption of myelin sheath of large myelinated nerve fibers is likely to contribute to the development of bilateral mechanical hyperalgesia and motor dysfunction.

P2-04-02

Passive diffusion of an RGD peptide decreases the responsiveness of slowly and rapidly adapting mechanoreceptors in rat skin

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During cutaneous mechanical stimulation, mammalian slowly and rapidly adapting mechanoreceptors (SAs and RAs, respectively) encode local stress or a stress-related quantity. The stress is distributed through the cutaneous extracellular matrix (ECM) and coupled to the cytoskeleton (CSK) of a given SA (or RA) via integrins. We previously found that function-blocking anti-integrin α2 monoclonal antibody (FBmAb) decreased the responsiveness of SAs and RAs in rat skin, suggesting the possible role of α2 integrins in modulating the deformation/opening of ion channels. However, the decrease of neural response may have been a result of the FBmAb–integrin binding itself. Since integrins bind to ECM proteins, specifically on arginine-glycine-aspartate (RGD), we reasoned that an RGD peptide diffused into the receptive fields of mechanoreceptors would compete with ECM proteins. Thus we hypothesize that disruption of integrins in cutaneous mechanoreceptors via RGD peptide will decrease their responsiveness to controlled mechanical loads. Skin and its intact innervation were harvested and stimulated using static or dynamic compressive loading before and after passive diffusion of an RGD peptide (GRGDSP sequence 25 μg/mL). The mean neural responses of SAs and RAs post-RGD decreased to 1.8% and 10.6%, respectively; subsequent to wash-out, the mean neural responsiveness was 80% and 105%, respectively. These data complement our prior results using anti-integrin α2 FBmAb and suggest that by either blocking integrin function or decoupling from the ECM results in decreases in neural response. These data support the concept that integrins play a substantive role in the mechanisms by which cutaneous mechanoreceptors encode stress.

P2-04-03

Rat model of pathological pain in the central nervous system

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Neuronal events leading to development of long-term potentiation (LTP) in the nociceptive pathways may be a cellular mechanism underlying central hyperalgesia. The objective was to investigate the supraspinal network involved in a model of spinal LTP after noxious stimuli. A noxious high-frequency conditioning stimulation (HFS) was given to the left sciatic nerve of female Sprague–Dawley rats to induce LTP. Field potentials were recorded from wide dynamic range neurons in the dorsal horn of the spinal cord. A clear LTP of the nociceptive transmission following HFS was observed for over 3 h. FDG PET dynamic scanswere performed after FDG injection. Data were MAP reconstructed with attenuation correction. Comparisons between rest and stimulation condition were performed with normalization to the global mean value of the brain. Increased activity in the somatosensory cortex was observed in the acute, but not the late group. In contrast, the late group exhibited an increase bilateral amygdala (contra > ipsilateral) and in midline periaqueductal grey (PAG). Decreased activity was in the acute group limited to the midline and ipsilateral dorsolateral pontine tegmentum (DLPT), and in the late group the extended bilaterally in DLPT in addition to a change in the rostroventral medulla (RVM). Further a relative increased activity was seen in cerebellum in the late group. At supraspinal level the HFS led in the acute group to activation in the somatosensory cortex. The brain regions involved in the late group (amygdala, PAG, DLPT and RVM) constitute a network of descending stress-induced analgesia, which apparently does not effectively inhibit the pathological activity in ascending spinal neurons.

P2-04-04

Elevation of interleukin (IL)-2, IL-6 and natural killercell activity induced by administration of peripheral electrical stimulation in human

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Acupuncture has been used in China and Asian countries for more than two thousand years. Peripheral electrical stimulation (PES) has been widely used as a substitute for classical acupuncture, and has effects on multiple physiological systems. PES is well known as one of the most popular forms of complementary medicine and has been proved to be an effective means for releasing pain and the treatment of certain chronic diseases. Our previous studies indicated that 2/100 Hz PES accelerates the release of endogenous opioid peptides in animals and humans, which produce analgesia via different type of opioid receptors. However, the mechanisms of the effects of 2/100 Hz PES on the immune function remain to be investigated, especially in human. Thus, the present study was designed to determine whether 2/100 Hz PES could modulate the immune function in human. The NK cell activity, cytokines level and phagocytic index represented the immune function in the present study. Healthy human was rendered to 2/100 Hz PES once a day for successive three days, the results showed that PES increased peripheral blood NK cell activity and phagocytic index significantly compared to that of pre-administration of PES, respectively ($p < 0.05$). Obviously and enhancement of interleukin-2 (IL-2) and IL-6, but not of IL-4, IL-10, IL-12, IFN-γ and IFN-α in serum were found after administration of PES ($p < 0.05$). Taken together, these results suggested that 2/100 Hz PES could modulate the immunological function in human, which might provide an effective means for the treatment of immunodeficiency and physical disorders.

P2-04-05

The relationship among serotoninergic terminals, 5-HT$_{1A}$ receptors, GABAergic interneurons and projection neurons in the ventrolateral orbital cortex of the rat

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Previous studies have indicated that the ventrolateral orbital cortex (VLO) is involved in an endogenous analgesic system consisting of the spinal cord-nucleus submedius (Sm) -VLO-periaqueductal gray (PAG)-spinal cord loop. Morphological results showed 5-HT immunoreactive (ir) neurons in the dorsal raphe nucleus (DRN) sent their axons to the VLO and behavioral study indicated that GABAergic modulation is involved in the VLO 5-HT$_{1A}$ receptor (R) mediated antinociception. In current study, we examined the relationship of 5-HTergic terminals from the DRN and GABAergic neurons expressing 5-HT$_{1A}$R and the neurons projecting to the PAG in the VLO of the rat by using anterograde and retrograde tracing combined with dual labeling or triple-labeling immunofluorescence histochemical and electron microscopic methods. Results showed that GABA-ir and 5-HT$_{1A}$R-ir neuronal cell bodies and terminals were distributed extensively throughout layers II-VI of the VLO; of these GABA-ir neurons, 93.0% showed 5-HT$_{1A}$R-immunoreactivity. In the VLO, the anterogradely labeled 5-HT-ir terminals from the DRN made close connections with GABA-ir neurons while the retrogradely labeled neurons expressing GABA$_A$R-immunoreactivity from the PAG made close connections with GABA-ir terminals. Under electron microscopy, majority of the GABA-ir neurons expressed 5-HT$_{1A}$ R-immunoreactivity and 5-HT-ir terminals made symmetrical synapses with GABA-ir terminals as well as GABA-ir terminals made symmetrical synapses with the retrogradely labeled neurons expressing GABA$_A$R-immunoreactivity from the PAG. These results provide direct morphological evidences that 5-HT may modulate the functions of GABAergic neurons in the VLO, via 5-HT$_{1A}$R, and lead to activate PAG-brain descending inhibitory system which induce antinociceptive effects.

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P2-04-06

Effects of electrical stimulation of the lateral hypothalamus on parabrachial nucleus gustatory responses in rats

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We have previously shown that stimulation of the rat central amygdaloid nucleus significantly affects taste responses in the parabrachial nucleus. The lateral hypothalamus receives projections from the parabrachial nucleus gustatory neurons and sends afferent projections to the parabrachial nucleus. Single unit recording was used to examine the effects of electrical stimulation of the lateral hypothalamus on taste neurons in the parabrachial nucleus. Among 30 taste neurons recorded, 60% were affected by lateral hypothalamus stimulation. During lateral hypothalamus stimulation, the responses of most affected parabrachial nucleus neurons were inhibited with the magnitude significantly lower than that obtained before stimulation ($p < 0.05$). Based on the best-stimulus category, the responses of the NaCl-best neurons to NaCl and HCl and the QHCl-best neurons to HCl and QHCl were significantly suppressed ($p < 0.05$). Analysis of across-unit patterns indicated that lateral hypothalamus stimulation decreased the correlations between NaCl and other stimuli, and increased the correlations between QHCl and other stimuli. These findings suggest that the lateral hypothalamus mediates feeding and taste via modulating the activity of parabrachial nucleus gustatory neurons.

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P2-04-07

Roles of 5-HT receptor subtypes in the effects of 5-HT on C-fiber responses of spinal wide dynamic range neurons in spinal nerve ligation rats

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5-Hydroxytryptamine (5-HT or serotonin) plays an important role in the descending control of nociception. 5-HT and its receptors have been extensively studied in the modulation of nociceptive transmission at the spinal level using behavioral tests that may be affected by the effects of 5-HT on motor performance and skin temperature. Using electrophysiological methods, the present study aims to systematically investigate the roles of 5-HT receptor subtypes on the inhibitory effects of 5-HT on responses of the spinal wide dynamic range (WDR) neurons to C-fiber inputs in spinal nerve ligation (SNL) rats. Under basal conditions, topical application of 5-HT to the spinal cord inhibited the C-fiber responses of WDR neurons dose-dependently, whereas antagonists of 5-HT$_{1A}$ (WAY 100635), 5-HT$_{1B}$ (GR 55562), 5-HT$_{2A}$ (ketanserin), 5-HT$_{2C}$ (RS 102221), 5-HT$_3$ (MDL 72222) and 5-HT$_4$ (GR 113808) had no effect on their own. The inhibitory effects of 5-HT were reversed by antagonists of 5-HT$_{2A}$ (ketanserin), but not by 5-HT1$_A$ (WAY 100635), 5-HT$_{1B}$ (GR 55562), 5-HT$_{2C}$ (RS 102221), 5-HT$_3$ (MDL 72222) and 5-HT$_4$ (GR 113808) receptor antagonists. Topical administration of agonists of 5-HT$_{1A}$ (8-OH-DPAT), 5-HT$_{2A}$ (α-m-5-HT) and 5-HT$_3$ (mCPBG) inhibited the C-responses. 5-HT$_{1B}$ (CGS 12066), 5-HT$_{2C}$ (MK 212) and 5-HT$_4$ (BZTZ) had no effect on the C-responses. These results suggest that under basal conditions, there is no tonic serotonergic inhibition on the C-responses of dorsal horn neurons and 5-HT$_{2A}$ receptor subtypes may be involved in mediating the inhibitory effects of 5-HT in SNL rats.

P2-04-08

Effects of induction of sodium appetite on the change of NaCl taste detection threshold of rats

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Sodium deficiency produces a motivated sodium appetite which is characterized by avid ingestion of the aversive highly concentrated

NaCl. Previous studies of mechanism of sodium appetite have suggested that it may be related to salt taste sensory changes. They were directed to the hypothesis that animals can increase the ability to ingest concentrated salt solutions to compensate for sodium deficiency. We aimed to determine whether salt taste detection threshold would change the ability to find sodium containing food or solutions when sodium appetite is induced in rats. Firstly, a conditioned taste aversion (CTA) was established to suprathreshold concentration of NaCl (0.1M), a series of two-bottle preference tests were conducted with one concentration each day. Low concentrations of NaCl solutions (ranging from 0.05M to 0.001M) were given to the rats. Results indicate that NaCl detection threshold lies between 0.003M and 0.005M. Then we tested NaCl detection threshold of rats with sodium appetite produced by diuretic furosemide to induce urinary sodium loss followed by low dose angiotensin-converting enzyme inhibitor captopril which is presumed to increase conversion of ANG II within circumventricular organs. We also tested with furosemide followed by high dose captopril which can block central ANG II synthesis. The rats using low-dose captopril can even detect 0.0003M NaCl, while NaCl detection threshold of rats injected with high-dose captopril lies between 0.003M and 0.005M, the same as that of normal rats. These results suggest sodium appetite induced by acute sodium loss may increase the taste ability to detect NaCl, and this effect may be related to the function of central rennin-angiotensin-aldosterone system.

P2-04-09

The analgesic effects of intrathecal administration of Ro 25-6981 on neuropathic pain in rats: study on the electrophysiological mechanism

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The potential mechanisms and the analgesic effects of intrathecal administration of Ro 25-6981, a selective antagonist of the NR2B subunit of NMDA receptors (NR2B), on neuropathic pain in rats were investigated. L_5 spinal nerve ligations (SNL) were performed after intrathecal catheterizations were succeeded in male Sprague-Dawley rats. The rats with neuropathic pain evaluated by von Frey filaments-induced mechanical allodynia were divided into three groups randomly. Ro 25-6981at the dose of 10 μg (20 μl) or 100 μg (20 μl), or saline solution at the same volume, was administrated to rats in each group by intrathecal injection. The 50% paw withdrawal thresholds (PWT) of the rats was tested with von Frey filaments at 30 min, 60 min, 90 min, and 120 min after Ro 25-6981 or saline solution administration. And the locomotor function was also examined by the slope board test at the same time points. The effects of Ro 25-6981 on the discharges of spinal wide dynamic range (WDR) neurons were examined through *in vivo* extracellular electrophysiological recording techniques in normal rats. Intrathecal injection of Ro 25-6981 at the dose of 10 μg had no significant effect on 50% PWT and %MPE in SNL rats as compared with that *i.t.* the same volume of saline solution ($p > 0.05$, $n = 8$); while at the dose of 100 μg showed significant increases in 50% PWT and %MPE ($p < 0.05$, $n = 8$). Moreover, no obvious impairment on locomotor function was observed after *i.t.* Ro 25-6981 at both of the above doses. Spinal administration of Ro 25-6981 at the dose of 100 μg had no significant effect on A_β-fiber evoked discharges of WDR neurons ($p > 0.05$, $n = 6$), but showed significant enhance in C-fiber evoked discharges ($p < 0.05$, $n = 6$). Intrathecal injection of Ro 25-6981 at the dose of 100 μg had significant analgesic effects on rats with neuropathic pain, but didn't affect their locomotor functions; it is likely that such analgesic effects were achieved via antagonizing the spinal NR2B subunit of NMDA receptors, and then inhibiting the C-fiber evoked discharges of spinal WDR neurons.

P2-04-10

The expression of DBH and AP-2α in the cerebellar purkinje cells and spinal cord of the painful rats with formalin-induction

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The changes of dopamine-β-hydroxylase (DBH) and activator protein 2-α (AP2-α) expression in spinal cord and cerebellar purkinje cells of normal and formalin induced pain model rats were investigated to explore mechanism of noradrenergic (NA) neuronal changes in the formalin induced pain model Wistar rats. Immunohistochemical (IHC), in situ hybridization, and immunohistochemistry were used to measure DBH/AP2-α protein and mRNA. After injection with formalin, the behavioral changes of rats appeared. A small number of DBH-positive neurons were sparsely distributed in ventral horn (10 ± 3), and a few in the dorsal horn. By day 3, many darkly-stained DBH-positive neurons appeared, predominately in both ventral horn (24 ± 3) and dorsal horn (33 ± 4). The staining density at day 7 was lower, but still higher than control. There existed a very significant difference in number and staining density of the NA neurons in painful spinal cord compared with that in the normal spinal cord ($p < 0.05$). The purkinje cells in both groups showed DBH-positive staining. However, the density in experimental groups were significantly increased ($p < 0.05$). Co-existence of DBH and AP2-α in the purkinje cells and the neurons in spinal cord was observed by means of immunohistomical double-labelling staining. The changes of AP2-α expression were similar to that of DBH in cerebellar purkinje cells and spinal cord in painful rats. Our data indicate that some non-NA neurons could convert into NA neurons in painful rats. NA may be involved in the formalin-induced pain and behavior regulation; as one of transcription factors, AP2-α may promote the DBH synthesis.

P2-05-01

The effect of etidocaine·HCL on the physical properties of neuronal membranes

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Fluorescent probe techniques were used to evaluate the effect of etidocaine·HCl on the physical properties (transbilayer asymmetric

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

P2-05-02

Intragastric administration of evodiamine reduces gene expression of NPY and AgRP in the hypothalamus and decreases food intake in rats

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Evodiamine, an alkaloidal component extracted from the fruit of *Evodiae fructus* (*Evodia rutaecarpa* Benth., Rutaceae), decreases body weight through a poorly defined mechanism. Hypothalamus is one of the areas in the brain linked to the control of food intake and energy expenditure. We postulate that evodiamine mediates this activity by modulating feeding-related peptides of the hypothalamus. We investigated the effects of evodiamine on food intake, body weight and the mRNA expression of hypothalamic neuropeptide Y (NPY), agouti-gene related protein (AgRP), melanin concentrating hormone (MCH), pro-opiomelanocortin (POMC), and melanocortin receptor-4 (MC4R) in male rats. Evodiamine (40 mg/kg or 4 mg/kg) was intragastrically administrated for 25 days, and the food intake and body weight of the rats were recorded daily. Blood samples were collected for leptin radioimmunoassay (RIA). Real-Time PCR was used to analyze mRNA expression. Our results show that intragastric administration of evodiamine (40 mg/kg) inhibits food intake, decreases body weight and reduces the mRNA expression of NPY and AgRP in the hypothalamus, while it increases the circulating level of leptin. This data indicate that evodiamine decreases food intake, and therefore body weight, partly by down-regulating the NPY and AgRP mRNA expression in the hypothalamus.

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P2-05-03

YY1 is the key regulator of the characterized murine faap gene promoter

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Focal adhesion associated protein (FAAP), a product of the *Mus musculus* D10Wsu52e gene, is highly conserved and ubiquitously expressed. RNA interference (RNAi) knockdown of C.elegans homologous F16A11.2 in resulted in male nervous system morphology abnormal. To understand the mechanisms of FAAP gene expression and regulation, we first mapped the major transcription start site at the nucleotide 79bp upstream of the ATG codon. Murine FAAP 2.1kb and human homologous HSPC117 645bp promoter regions were cloned and analyzed. Truncation analysis of promoters identified minimal 0.2kb efficient TATA-less promoter regions. The substantive repeat sequence of the FAAP gene promoter greatly inhibited its transcription activity. Testis *in vivo* electroporation confirmed the activity of the identified murine promoter. Electrophoretic mobility shift assays validated the specific interaction between YY1 and the predicted element in murine minimal region. Site mutagenesis and knockdown of YY1 indicated that YY1 was crucial to FAAP gene promoter activity; while truncation analysis and overexpression of YY1 suggested that the function of YY1 was related to upstream elements. Furthermore, during embryogenesis, the expression patterns of FAAP and YY1 were similar. Our data demonstrated that YY1 was an important regulator of the characterized FAAP gene promoter.

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P2-05-04

Tau protein binding to the minor groove of DNA double-stranded

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Tau, an important microtubule associated protein, has been found to bind to DNA, and to be localized in the nuclei of both neurons and some non-neuronal cells, associating with nucleolar organizer regions (NORs) of acrocentric chromosomes. Here, using electrophoretic mobility shifting assay (EMSA) in the presence of DNA with different chain-lengths, we observed that tau protein favored binding to a 13-bp or a longer polynucleotide. The results from atomic force microscopy also showed that tau protein preferred a 13-bp polynucleotide to a 12-bp or shorter polynucleotide. In a competitive assay, a minor groove binder distamycin A was able to replace the bound tau from the DNA double helix, indicating that tau protein binds to the minor groove. In the presence of DNase I, tau protein protected double-stranded DNA from digestion. This also suggests the DNA minor groove as the binding site for tau because DNase I recognizes the minor groove. EMSA with truncated tau proteins showed that both the proline-rich domain (PRD) and the microtubule-binding domain (MTBD) contributed to the interaction with DNA; that is to say, both PRD and MTBD bound to the minor groove of DNA and bent the double-strand, as observed by electron microscopy. Tau protein appears to interact with DNA in a chaperone-like manner.

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P2-05-05

MRI/MRS of ischemic evolution in mouse brain at 14.1 Tesla

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Magnetic resonance (MR) imaging (MRI) and spectroscopy (MRS) are ideal tools suited to study ischemic evolution events. Mouse studies are common to help understand the pathogenesis but remains challenging due to its organ size, especially in MRS. High magnetic fields increase sensitivities and thus using a recently installed 14.1 T/26cm MR system, we sought to determine the feasibility of studying lesion development and neurochemical changes following 30 min of endoluminal middle cerebral artery occlusion in four iCR-CD1 mice using filament techniques. Absolute rCBF dropped to 21 ± 6% of control during ischemica and recovered back to 70 ± 24% shortly after. 8 and 24h after the insult, T_2-weighted images presented lesion developing and localized spectra were obtained with 0.035ppm linewidths in the stroke region after adjusting magnetic field inhomogeneities. The resulting signal-noise-ratio allows reliable analysis of 18 metabolites from an *in vivo* spectrum using a linear combination of model spectra of metabolites. Prominent changes at 8 h ischemia include a transient doubling of brain Gln to 6.9 ± 1.3mM, postulated to reflect Glu excitotoxicity, and decreases in several compounds: NAA (3.6 ± 1.2mM), Glu (5.0 ± 1.0mM), tau (5.0 ± 1.0mM), as well as increases in others acetate 0.4 ± 0.2mM (attributed to NAA breakdown) and lactate (10.0 ± 2.3mM). Ischemia results in profound changes in the neurochemical profile with a complex evolution pattern, such as the Gln/Glu ratio, from 1.4 at 8 h to 0.4 at 24 h. Even at 8 h, the significant abnormality in the neurochemical profile was observed consistently when T_2-weighted images presented minor lesion. We conclude that both MRI and MRS are feasible in ischemic mouse brain at 14.1T.

P2-05-06

In vivo human brain γ-aminobutyric acid detection by 1H MRS at 3.0 T with the J-Editing technique: sensitivity enhancement, macromolecule contamination and test-retest reliability

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Measuring brain levels of γ-aminobutyric acid (GABA) is of great interest due to its potential involvement in the pathophysiology of most neuropsychiatric and many neurological disorders. However, detection of brain GABA by ^{1}H MRS presents formidable challenges due to its low concentration, the overlap of its resonances, and contamination of the detected signal by mobile macromolecule (MM) signals. To overcome these impediments and obtain reliable brain GABA measurements using the standard J-editing difference technique at 3.0 T by evaluating detection sensitivity gains that can be attained with an 8-channel head coil, and estimating the magnitude and extent of anatomic variation of the contamination of GABA by MM. Sensitivity gains were assessed for a voxel in the dorsolateral prefrontal cortex (DLPFC), whereas MM levels and the degree of contamination were assessed for voxels in the DLPFC, the anterior cingulate cortex (ACC) and the occipital cortex (OCC)..We achieved two fold increases in sensitivity with the 8-channel head coil over a standard one-channel head coil, and allowed the scan time to be shortened or voxel size to be decreased by 50% without degrading spectral quality. Assessing the levels of total GABA (i.e., GABA+MM) and MM in DLPFC, OCC and ACC showed significant anatomic variation across the three regions ($p < 0.05$). However, the contribution of MM to total GABA was relatively stable across the three voxels, ranging from 41% to 49%, a non-significant regional variation In conclusion an 8-channel head coil at 3.0 T can enhance the ease and reliability of human brain GABA detection by ^{1}H MRS.

P2-05-07

Investigation of the change of neurotransmitter after different time of rat death

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Here we investigate the change between the power of neurotransmitter and entropy under different doses of anesthetization. During the gradual increase of the degree of anesthetization, we study the change using an encephalofluctuograph. As anesthetization increased so the power of six neurotransmitters, gamma-amino butyric acid, glutamate, 5-hydroxytryptamin, acetylcholine, noradrenaline, dopamine, did gradually decrease. Entropy also shows a gradual increase, suggesting brain function gradually decreases with the increasing degree of anesthetization. The power and entropy of neurotransmitters are reflected by the changing degree of anesthetization.

