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Poster Abstracts

P01

Effects of 3 and 6 months guanosine treatment on cognitive impairment of rats submitted to chronic cerebral hypoperfusion and chronich (only 6 months) treatment on hippocampal damage

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Chronic cerebral hypoperfusion contributes to a cognitive decline related to brain disorders. Its experimental model in rats is a permanent bilateral common carotid artery occlusion (2VO). Overstimulation of the glutamatergic system excitotoxicity due to brain energetic disturbance in 2VO animals seems to play a pivotal role as a mechanism of cerebral damage. Several studies support the hypothesis that nucleoside guanosine (GUO) exerts extracellular effects including antagonism of glutamatergic activity. It has been reported that GUO is able to reduce glutamatergic activity, since it was shown that GUO inhibits the binding of glutamate and its analogs to brain membrane preparations, to prevent cell responses to glutamate and to stimulate glutamate uptake by cultured astrocytes. In this study we therefore evaluate chronic GUO treatment effects (cognitive and hippocampal damage) in rats submitted to 2VO experimental model. We assayed the performance of animals in the Morris water maze and hippocampal damage by neurons and astrocytes by immunohistochemistry. Additionally, we investigated the cerebrospinal fluid (CSF) brain-derived neurotrophic factor (BDNF) and serum S100B levels. Finally, the purine CSF and plasma levels were determined. Our results show that at both 3 and 6 months, GUO treatment did not prevent the cognitive impairment promoted by 2VO. However, none of the 2VO animals treated with GUO showed differences in the hippocampal regions compared to control, while 20% of 2VO rats not treated with GUO presented loss of pyramidal neurons and increased glial labeling cells in CA1 hippocampal region. In addition, we did not observe differences in CSF BDNF or serum S100B levels among the groups. Of note, both the 2VO surgery and GUO treatment changed the purine CSF and plasma profile. In conclusion, GUO treatment did not prevent the cognitive impairment observed in 2VO animals, but our data suggest that GUO could be neuroprotective against hippocampal damage induced by 2VO.

P02

Neuronal glycoprotein M6a induces filopodium formation through a GIT1/Rac1 pathway

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Gpm6a codes for neuronal membrane glycoprotein M6a, a member of the proteolipid protein family (PLP/DM20) whose role in neurite and filopodium formation has been demonstrated recently. *Gpm6a* was identified as a stress-responsive gene down-regulated in the hippocampus of chronically stressed animals. This effect was reversed by antidepressants. The consequences of chronic stress and depression, and the mechanisms of neuroplasticity and antidepressant response have been shown to converge on an overlapping set of events. Various studies have demonstrated that chronic stress causes reduction in dendritic arborizations and a loss of highly specialized dendritic spines in the hippocampus of primates and rodents. We hypothesize that reduced expression of *gpm6a* might be responsible for some of the morphological alterations found in the hippocampus of chronically stressed animals. The molecular mechanism that would explain how M6a regulates neurite and filopodium outgrowth and its involvement in chronic stress response remains unclear. In the present study we analyzed a possible signaling pathway by which M6a regulates filopodium formation. Coimmunoprecipitation followed by mass spectrometry revealed G protein-coupled receptor kinase-interacting protein 1 (GIT1) as a potential M6a interacting partner. GIT1 is a multidomain adaptor protein. One of its functions is to regulate spine morphogenesis and synapse formation by targeting actin regulators and locally modulating Rac activity. Here, the effect of the coexpression of wild type (wt) and mutant form of GIT1-GFP with wt M6a-RFP was analyzed in hippocampal neurons in culture using fluorescent microscopy. The coexpression of both forms of GIT1 with M6a prevented an increase in filopodium density induced by M6a. Overexpression of a dominant negative form of Rac1 that functions downstream of GIT1 also impaired an increase in filopodium density induced by M6a. Based on our data, we suggest that the function of M6a in filopodium formation in neurons requires local regulation of Rac1 activity through GIT1. We propose that the GIT1/Rac1 signaling pathway represents a link between actin cytoskeleton regulation and the function of M6a in filopodium outgrowth.

P03

Nicotine prevents synaptic impairment induced by Aβ oligomers through α7-nicotinic ACh receptor activation

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An emerging view on Alzheimer disease's (AD) pathogenesis considers amyloid-β (Aβ) oligomers as a key factor in synaptic impairment and memory decline. Alterations of the α7-nicotinic acetylcholine receptor (α7-nAChR) have been implicated in AD pathology. In this study we used nicotine, as an agonist of the α7-nAChR to improve the synaptic failure in culture hippocampal neurons treated with Aβ oligomers and in a transgenic model of AD.

We report here that treatment with nicotine in 5-month-old transgenic APP_{swe}/PS1ΔE9 mice (early state of pathology) shows a significant improvement in the Morris water maze test compared to control animals. Even in one-year-old transgenic mice (advanced state of pathology), nicotine improves the performance in the Morris water that measures spatial memory.

Treatment of cultured hippocampal neurons with Aβ oligomers for 1 h produces a postsynaptic impairment, resulting in a decrease in PSD-95 cluster density in dendritic spines, as well as in the number of synaptic contacts per neurite length. These effects were prevented by co-incubation with nicotine. In longer exposures to Aβ oligomers for 24 h, there is also presynaptic damage as evidenced by the decrease in synapsin I clusters. This latter effect was prevented by co-treatment with nicotine. Using specific signaling inhibitors, we show that the effect of nicotine in the prevention of

in both serotonergic and dopaminergic signaling. Cerebral hemispheres were dissected and cut into 400 μm slices using a tissue chopper. Accumulation of cAMP was measured in these slices after brief stimulation with serotonin, dopamine, SKF -38393 (D1-receptor agonist), SCH- 23390 (D1-receptor antagonist) or Raclopride (D2-receptor antagonist). We detected differences between genotypes in response to both neurotransmitters. Serotonin-mediated responses were about 30% greater in KO animals, when compared with WT controls. Dopamine, on the other hand, produced a response approximately 40% greater in WT animals than in PrPc-null tissue. With the use of dopaminergic agonist and antagonists we found that the distinct dopaminergic responses can be attributed to D1-like receptors only, while D2-like receptor responses appeared to be unrelated to the differences between WT and KO mice. Using high performance liquid chromatography (HPLC), we found that serotonin levels, as well as of its metabolite (5-HIAA) were similar in both genotypes, while dopamine levels were 3-fold larger in KO than in WT animals. Dopamine metabolites (HVA and DOPAC) showed, however, no differences between the distinct genotypes. These results indicate that PrPc modulates the balance between the levels of these two neurotransmitters in the cerebral cortex, and suggest an important role for PrPc in monoaminergic synaptic transmission.

P07

Role of astrocytes in response to hypoxia in developing central nervous system

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Hypoxia, which occurs in the central nervous system (CNS) when oxygen availability drops below the normal level, is a major cause of prenatal hypoxic-ischemic injury. The transcription factor Hypoxia Inducible Factor-1 (HIF-1) is a key regulator of oxygen homeostasis and is stabilized by insults associated with hypoxia and ischemia. Because its many target genes mediate both adaptative and pathological process, the role of HIF-1 during hypoxic injury is a matter of debate. Previous work in our model of acute hypoxia (60 min, 8% of O_2) in chicken embryos has shown that in the optic tectum (OT), hypoxic cell death is delayed after the end of the hypoxia treatment. We also found a stabilization of HIF-1 after the hypoxic stimuli, which rapidly decreases during the first minutes of reoxygenation. Preliminary results have shown the increase in the level of HIF-1 mRNA to be delayed after the end of the hypoxic injury.

Although HIF-1 is expressed at higher levels in neurons than astrocytes, HIF-1 induces multiple astrocyte-specific targets during hypoxia, implicating a role for HIF-1 in astrocytes as well as neurons. We aimed to study the role of astrocytes during the hypoxic insult both *in vivo* and *in vitro*. For this purpose, we first characterized the presence of mature astrocytes in chicken embryos in different embryonic days (ED). Western Blot studies demonstrated that the level of GFAP (glial fibrillary acid protein) expression was first appreciable at DE14 (ANOVA test, $p < 0{,}001$), and was increased thereafter. For our experiments, we designated ED15 as a representative day where mature astrocytes exist. We analyzed the levels of GFAP in our model of acute hypoxia. Western Blot studies demonstrated a six-hour delay in the increase in the level of GFAP after the end of the hypoxia treatment, showing a response to hypoxia by astrocytes. We also demonstrated that cultured astrocytes are vulnerable to acute hypoxia.

Preliminary results show that HIF-1 is stabilized in cultured astrocytes after hypoxia. In conclusion, this study reveals a role of astrocytes in developing CNS during hypoxic insult; however further experiments are necessary to explain and elucidate this role.

P08

Cholesterol exerts different modulatory effects at the cell surface of muscle and neuronal nicotinic acetylcholine receptors

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Communication at synapses requires the location and maintenance of receptors at specific sites. Factors controlling the distribution of receptors are critical determinants of the cell response to external signals. The nicotinic acetylcholine receptor (AChR) is the best characterized ligand-gated ion channel, found at neuromuscular junctions and central nervous system synapses. We have reported that cholesterol contributes to the homeostasis of muscle-type receptor levels at the plasmalemma (*Kumari et al., J. Cell Biol., 2008; Borroni and Barrantes, J. Biol. Chem., 2011*). Cyclodextrin-mediated acute cholesterol depletion lowered the number of cell-surface muscle AChRs in CHO-K1/A5 cells and C2C12 muscle cells. In contrast, the same treatment, performed on cultured hippocampal neurons, increased the amount of α7 AChR at the plasmalemma. In addition, chronic cholesterol reduction mediated by Mevinolin, an inhibitor of cholesterol biosynthesis, augmented the cell-surface levels of α7 AChR and α4β2 neuronal AChR subtypes measured by fluorescence microscopy and radioactive ligand binding assays. The results suggest that cholesterol modulates surface AChR levels differentially in peripheral and central cholinergic synapses.

P09

Induction and localization of heat shock proteins in cortical cultures following treatment with celastrol

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Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (ALS), are 'protein misfolding disorders' of the nervous system that are characterized by accumulation of protein aggregates and selective cell loss. Different brain regions are impacted, with Alzheimer's affecting cells in the cerebral cortex, Parkinson's targeting dopaminergic cells in the substantia nigra and ALS exhibiting degeneration of cells in the spinal cord. These neurodegenerative diseases differ widely in frequency in the human population. Alzheimer's is more frequent than Parkinson's and ALS. Heat shock proteins (Hsps) are 'protein