Journal of Neurochemistry JNC

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

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Anti-dyskinetic potential of the neuronal nitric oxide synthase inhibitor 7-nitroindazole is associated with reduction of striatal overexpression of FosB/DeltaFosB

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Repeated treatment with L-DOPA induces dyskinesia in rats with unilateral 6- OHDA-induced lesions of dopaminergic pathways in the medial forebrain bundle. These effects were prevented by 7-nitroindazole (7-NI), a preferential inhibitor of neuronal nitric oxide synthase, at doses that did not interfere with the positive motor effects of the L-DOPA. The aim of the present study was to verify whether acute and/or chronic administration of 7-NI modifies striatal expression of the transcription factor FosB/DeltaFosB, an indicator of persistent cellular activation. Male Wistar rats with unilateral 6-OHDA lesions of the medial forebrain bundle or sham-operated animals (n = 5–7/group) were treated chronically (21 days) with L-DOPA (30 mg/kg plus benserazide 12.5 mg/kg) to induce abnormal involuntary movements (AIMs). Over the course of L-DOPA treatment, the rats developed AIMs classified as axial, limb, orofacial and locomotive dyskinesia. Both acute (single dose) and chronic (daily) administration of 7-NI (30 mg/kg, i.p.) 30 min prior to L-DOPA strongly reduced the severity of AIMs. Also, rats co-treated with 7-NI and L-DOPA exhibited an improvement in the stepping test, showing that the blockage of AIMs was not due to a reduction in the general motor activity. 7-NI significantly attenuated the overexpression of FosB/Delta-FosB associated with L-DOPA-induced dyskinesia and this effect was more pronounced in lateral portions of striatum. The findings here may represent a new approach to the management of L-DOPA-therapy in Parkinson's disease treatment. Financial support: The State of Sao Paulo Research Foundation (FAPESP).

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Endocannabinoid 2-AG metabolism in rat cerebral cortex during physiological aging

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The ''cannabinoid system'' is a cell communication mechanism which involves the interaction of endogenous ligands, membrane receptors, and signal inactivation processes. 2-arachidonoilglycerol (2-AG), which is synthesized and released in response either to an increase in intracellular calcium or to the action of metabotropic agonists, is one of the endogenous ligands of cannabinoid receptors CB1 and CB2 that mediate its signalling coupled to G proteins. The enzymes responsible for its synthesis are diacylglycerol lipase (DAGL) and lysophosphatidate phosphohydrolase (LPAase). Its hydrolysis is carried out principally by the enzyme monoacylglycerol lipase (MAGL), although other enzymes may be involved in its breakdown such as fatty acid amide hydrolase (FAAH) and serine hydrolase ABHD. Although it is well known that endocannabionoids play a role as neuroprotectors in pathological senescent processes, their role in physiological senescent processes has not been fully elucidated to date. We thus suggest that 2-AG synthesis and hydrolysis enzymes both of which control its level, could be regulated in physiological aging. To approach this hypothesis we firstly characterized the enzymatic activities involved in 2-AG synthesis and hydrolysis in membrane, soluble and synaptosomal fractions from adult (3 months) and aged (28 months) rat cerebral cortex (CC). CC fractions were isolated by differential centrifugation and synaptosomes were purified in ficoll gradients. DAGL, MAGL and LPAase activities were assayed using tritium radiolabeled substrates, and their products monoacyl[^{3}H]glycerol y [^{3}H]glycerol were quantified by liquid scintillation from organic or aqueous phase, respectively. Our observations showed that: (i) LPAasa activity is the most active pathway for 2-AG synthesis; (ii) there is a decrease in LPAase activity and a redistribution of DAGL activity from the soluble to the membrane fraction as a result of aging; (iii) 2-AG hydrolysis in adult membrane is carried out by ABHD and by MAGL while ABHD is the only enzyme responsible for cannabinoid hydrolysis in aged membrane; (iv) DAGL activity is low while LPAase activity is high in aged synaptosomes; (v) MAGL, FAAH and ABHD are responsible for 2-AG hydrolysis in adult synaptosomes; (vi) MAGL is responsible, almost exclusively, for 2-AG hydrolysis in aged synaptosomes. Results from the present study reveal a precise regulation of 2-AG metabolism, which is, in turn, modified in physiological aging.

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Membrane proteomic analysis of rat nucleus accumbens following self-administration of cocaine, heroin and cocaine/heroin, ''speedball,'' combinations

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The concurrent use of cocaine with opiates, ''speedball,'' represents a growing subset of drug users, possibly due to enhanced yet distinctively subjective and euphorigenic effects of the combination. Speedball is abused in a variety of different patterns and exerts significant effects on mental and physical health, social adjustment and outcome of opiate-addiction treatment. Unfortunately, the neurobiological substrates mediating the abuse of speedball remain unclear. Previously, our lab has demonstrated that speedball self-administration induces a synergistic increase in dopamine neurotransmission in the nucleus accumbens (NAc) compared to either drug alone, an effect that may contribute to the behavioral and neurological pathology observed in speedball abusers. Preclinical studies support the concept of pharmacological uniqueness of the combination, suggesting that current treatment options for cocaine or heroin abuse may not be effective for treating speedball abuse. The present study was undertaken to further understand the neuropathology of chronic speedball use by comparing the NAc synaptic proteomes from rats self-administering cocaine, heroin and speedball to controls following chronic self-administration. Briefly, peptides generated from trypsin digestion of membrane protein fractions were labeled using isobaric mass labeling (iTRAQ 8plex). Labeled peptide samples from all groups were combined and then separated by strong cation exchange and reverse phase liquid chromatography and spotted onto MALDI plates for analysis by MALDI-TOF/TOF