0271 - AMPHETAMINE INDUCED DIFFERENTIAL EFFECTS IN VASCULAR AND GLIAL COMPONENTS AT SOMATOSENSORY CORTEX: WHY TO FOCUS ON AT1 RECEPTORS

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Amphetamine (Amph), is associated with inflammatory processes, involving glial and vascular alterations. Brain Angiotensin II, through AT1-receptors (AT1-R), modulates dopaminergic neurotransmission and plays a crucial role in inflammatory responses. Our aim was to evaluate the role of AT1-R in long-term alterations induced by repeated exposure to Amph. Astrocyte and microglia reactivity, and brain microvascular network were analysed at the somatosensory cortex (S1 Barrel and S1 Trunk area). Male Wistar rats (250–320 g) were administered with AT1-R antagonist Candesartan/vehicle (3 mg/kg p.o., days 1–10) and Amph/saline (2.5 mg/kg i.p., days 6–10). The four experimental groups at the two times evaluated (17 and 31 days) were: Veh-Sal, CV-Sal, Veh-Amph, CV-Amph. On days 17 and 31 the animals were sacrificed and their brains were processed for immunohistochemistry against GFAP (astroglial marker), CD11b (microglial marker) and von Willebrand factor (vascular marker). Data were analysed with factorial ANOVA followed by Bonferroni test. Our results indicate that Amph exposure induces an endurable increase in astrocyte and microglia reactivity at S1 Barrel and S1 Trunk area. Although, the microvascular rearrangement (evaluated as vascular area density, branching points and tortuosity) showed time dependant differential response to Amph, since at day 31 these parameters return to basal conditions at S1 Barrel. Meanwhile, at S1 Trunk the vascular changes were observed only at day 31. Pretreatment with the AT1-R blocker prevented the described alterations induced by Amph. We conclude that neuroplastic changes induced by Amph demand an AT1-R active role showing a regional susceptibility at vascular level.

0289 - ROLE OF PRO-INFLAMMATORY FACTORS ON THE SURVIVAL AND DIFFERENTIATION OF DOPAMINERGIC PRECURSORS

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Parkinson's disease (PD) is a neurodegenerative disease characterized by the progressive loss of dopaminergic neurons (DAn) of the nigrostriatal system. Studies in animal models of PD have provided proof of concept that transplantation of DA precursors can relieve parkinsonian symptoms. However, a major limiting factor of this strategy is the poor survival rate of grafted DAn. This could be due to host inflammatory response, among other factors. Our previous results demonstrated a host primary response related to the graft of human dopaminergic precursors (DA14), with an increase of host-MHCII positive cells. Expression of tumor necrosis factor-alpha (TNF-alpha) was also detected on host-ED1 positive cells. We aim to study the impact of the pro-inflammatory environment on DA14 cells and the effect of a TNF-alpha inhibitor on an in vitro approach. DA14 cells were exposed to conditioned media (CM) from basal or activated BV2 microglial cells during 4 days. A significant increase in cell death was observed by fluorescence microscopy after Hoechst staining in DA14 cultures exposed with CM from activated microglia ($p<0.05$). This result was in accordance to a decrease in the number of Tyrosine hydroxylase (TH) positive cells detected by immunofluorescence ($p<0.01$). Neurite length measurement was performed to evaluate the differentiation process. A decrease in neurite length of TH positive cells was detected in DA14 cultures incubated with CM from activated microglia ($p<0.05$). In order to study the relevance of TNF-alpha, DA14 cells were co-incubated with CM from basal or activated BV2 cells and the TNF-alpha inhibitor, Etanercept. Inhibition of TNF-alpha was able to avoid morphological alterations ($p<0.05$) and diminution of DA cells ($p<0.05$). Our results suggest that the pro-inflammatory microenvironment has a negative impact on survival and differentiation of DA14 cells. TNF-alpha inhibition could be an interesting strategy in order to improve survival of DA precursors.

0393 - NEW HYPERGLYCOSYLATED HUMAN ERYTHROPOIETIN-DERIVED MOLECULES AS THERAPEUTIC CANDIDATES FOR THE TREATMENT OF THE CENTRAL NERVOUS SYSTEM-AFFECTING DISEASES

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Neurodegenerative diseases affect millions of people around the world causing cognitive and behaviour disorders. Nevertheless, does not exist any effective treatment for them nowadays. In this sense, human erythropoietin (EPO) has an important role considering its antiapoptotic, cytoprotective, angiogenic and antioxidant properties. However, its erythropoietic activity (EA) should be considered as a side effect. Thus, we proposed the development of new EPO analogues using an approach of N-glycoengineering by hyperglycosylation to preserve its neuroprotective and neuroplastic action but blocking the EA. New EPO muteins were obtained by adding one extra N-glycosylation site per molecule using site–directed mutagenesis. Then, they were produced in transduced CHO.K1 cells and purified by immunoaffinity chromatography. Primary cultures from hippocampal neurons were used to measure apoptosis inhibition, neuritogenesis, filopodia density and synapsis formation. In vitro and in vivo EA was also carried out. Three EPO variants were produced and one-step purified with a purity level higher than 89%. They presented an apparent molecular mass higher than EPO and a superior number of acidic isoforms as result of the increased glycosylation degree. The in vitro and the in vivo haematological activity of each EPO analogue was abolished ($p<0.001$). Nevertheless, all of them preserved the neuroprotective and neuroplastic activity as they prevented staurosporine-induced apoptosis ($p<0.001$) but promoted neuritogenesis ($p<0.05$ and $p<0.001$), filopodia density ($p<0.05$ and $p<0.001$) and synapsis formation ($p<0.01$ and $p<0.01$). Thus, blocking the erythropoiesis-stimulating activity of hEPO and retaining its neuroprotective and neuroplastic action, potentiate these new hyperglycosylated EPO entities as novel neurobiopharmaceutics useful for the treatment of those diseases affecting the central nervous system.

Infectología y Parasitología / Infectology and Parasitology II

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0043 - TRYPANOSOMA CRUZI PROLINE PERMEASE HAS A UNIQUE POLYAMINE CO-TRANSPORT ACTIVITY