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BDNF trafficking and signaling impairment during early neurodegeneration is prevented by moderate physical activity

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ABSTRACT

Physical exercise can attenuate the effects of aging on the central nervous system by increasing the expression of neurotrophins such as brain-derived neurotrophic factor (BDNF), which promotes dendritic branching and enhances synaptic machinery, through interaction with its receptor TrkB. TrkB receptors are synthesized in the cell body and are transported to the axonal terminals and anchored to plasma membrane, through SLP1, CRMP2 and Rab27B, associated with KIF1B. Retrograde trafficking is made by EDH-4 together with dynactin and dynein molecular motors. In the present study it was found that early neurodegeneration is accompanied by decrease in BDNF signaling, in the absence of hyperphosphorylated tau aggregation, in hippocampus of 11 months old Lewis rats exposed to rotenone. It was also demonstrated that moderate physical activity (treadmill running, during 6 weeks, concomitant to rotenone exposure) prevents the impairment of BDNF system in aged rats, which may contribute to delay neurodegeneration. In conclusion, decrease in BDNF and TrkB vesicles occurs before large aggregate-like p-Tau are formed and physical activity applied during early neurodegeneration may be of relevance to prevent BDNF system decay.

1. Introduction

Alzheimer's disease (AD) is the most common cause of dementia, characterized by progressive neurodegeneration. AD is characterized by the presence of amyloid-beta (Aβ) deposits in the form of senile plaques and the intraneuronal aggregation of hyperphosphorylated tau, a microtubule-associated protein, in hippocampus (Schindowski et al., 2008; Simic et al., 2016). These aggregates seem to be associated with a variety of cellular insults like synaptic failure, dysregulation of intracellular signaling cascades, disruption of axonal transport, oxidative stress and mitochondrial dysfunction (Hashimoto et al., 2003). However, it remains uncharacterized whether protein aggregation is the direct cause of neuronal death or there are cellular events that occur in the absence of protein aggregated and collaborate for cell damage.

It is well accepted that oligomers, which are the early stages of protein aggregation, might be more toxic than protein inclusions, since they may disrupt axonal transport as well as spread tau oligomeric seeds (Chung et al., 2001; Hoover et al., 2010; Lasagna-Reeves et al., 2010; Flach et al., 2012; Xie and Chung, 2012; Riemer and Kins, 2013).

Maintenance of neuronal function, neuroplasticity, morphogenesis and survival are ensured by axonal transport of vesicles, mitochondria, neurofilaments and other cell components (Decker et al., 2010; Hirokawa et al., 2010). Thus dysregulation of trafficking has significant physiological consequences in neurons, such as synaptic dysfunction. Moreover, early stages of AD are characterized by disruption in axonal transport, corroborating with the disease pathophysiology (Muresan and Muresan, 2009; Goldstein, 2012; Millecamps and Julien, 2013).

Several previous studies demonstrated decreased spine growth, synaptic transmission, loss of memory and learning difficulties by disruption on transport of brain-derived neurotrophic factor (BDNF) and its receptors (Kennedy et al., 2010; Lu et al., 2013; Yoshii et al., 2013).

BDNF is the major brain neurotrophin. It is involved in neuronal differentiation, maturation and survival; it modulates synaptic transmission and neuronal plasticity (Sebastiao et al., 2011). Intracellular signaling of BDNF involves activation of tropomyosin-related kinase B (TrkB) receptor, which may be associated to a

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tyrosine kinase intracellular domain (TrkB-full length; TrkB-fl) or independent of kinase activity (TrkB-truncated; TrkB-tc), and activation of PLCγ, PI3K/Akt, and ERK/MAPK pathways (Friedman, 2000; Patapoutian and Reichardt, 2001; Numakawa et al., 2010; Luine and Frankfurt, 2013; Kellner et al., 2014).

BDNF and TrkB-fl receptors levels decrease during aging (Webster et al., 2006; Diogenes et al., 2007; Lee et al., 2009), in AD their levels are further reduced (Ginsberg et al., 2006; Kao et al., 2012). Therefore, impaired transport and low expression of BDNF might contribute to synaptic dysfunction in AD (Khatri and Man, 2013; Scharfman and Chao, 2013). However, it remains poorly understood whether impaired axonal transport is cause or consequence of reduced BDNF signaling, and whether this is affected during the course of protein aggregation.

In neurons, anterograde trafficking of TrkB receptors is done by the interaction among Slp1, Rab27B and CRMP-2 proteins that direct link TrkB to the molecular motor Kinesin-1 (Arimura et al., 2009; Hirokawa et al., 2010). For retrograde trafficking, Pincher (EDH-4) and Rab5 are proteins that interact with the dynein/dynactin complex (Valdez et al., 2005; Zweifel et al., 2005; Hirokawa et al., 2010; Philippidou et al., 2011).

Physical exercise is postulated to promote neurogenesis through increase in BDNF levels, as well as increasing the levels of some proteins involved in axonal trafficking (Molteni et al., 2002; Ploughman, 2008; Erickson et al., 2012). Furthermore, physical exercise prevents other common features of aging, such as hippocampal atrophy, loss of memory and depression (Ogonovszky et al., 2005; Webster et al., 2006; Diogenes et al., 2007; Luellen et al., 2007; Lee et al., 2009; Marais et al., 2009; Erickson et al., 2012). However, the importance of physical exercise and the role of early protein aggregation in modulating axonal transport of BDNF and TrkB receptors during ageing and neurodegeneration are not well understood.

In view of this, it is hypothesized that BDNF levels, trafficking and signaling are disrupted in early neurodegeneration and physical exercise might interfere in this system.

2. Material and methods

Experiments were conducted in agreement with the International Guideline for Animal Experimentation care and use (Demers et al., 2006), and the Brazilian federal animal welfare law 11794/08. Procedures were approved by the research ethics committee (CEUA 121/11 and 451/11) of the Institute for Biosciences, University of Sao Paulo.

2.1. Animals, rotenone exposure and physical exercise

Twenty aged male Lewis rats (9 months old), supplied by the central animal facility of the Institute of Biosciences of the University of Sao Paulo, were housed in groups of 3–4 animals per conventional cage, maintained at 23 C ± 2, in an inverted 12 h light/12 h dark cycle (lights off at 6 a.m.), with free access to food and water.

Animals had osmotic minipumps (Alzet) implanted subcutaneously, between their scapulae, under anesthesia with ketamine (1.25 ml/kg) and xylazine (0.5 ml/kg). Minipumps were filled either with rotenone (Sigma, USA) dissolved in equal volumes of dimethyl sulfoxide (DMSO, Sigma, USA) and polyethylene glycol (PEG, Sigma, USA) which was delivered at the rate of either 1 mg/kg/day during 8 weeks (n = 10 animals), or only DMSO:PEG (1:1, n = 10 animals) as control. Minipumps were replaced at week 4 to guarantee 8 weeks of treatment.

One week after minipumps implant, all Lewis rats were familiarized to treadmill during 3 weeks, 3×/week, 30 min/day. After this, rats were preselected according to their ability to run in a treadmill and allocated to aerobic training (EXE, 50–60% of maximal exercise capacity, 5 days/week, 40 min/day, during 6 weeks) or kept sedentary (SED).

Maximal exercise capacity was determined by the maximal exercise test (starting at 0.3 km/h, with increments of 0.3 km/h every 3 min until exhaustion), which was repeated every 2 weeks in order to maintain training intensity.

Those rats that did not run or stopped running during protocol were excluded from the analysis. Rats were then divided in 4 groups (n = 5): DMSO-SED, DMSO-EXE, ROT-SED and ROT-EXE.

After treatment, animals were euthanized and their hippocampus removed and stored at −20 °C in protein extraction buffer (400 μl of PBS, pH 7.4, containing 1% NP40, 0.5% sodium deoxycholate, 1% SDS, 1 mM EDTA, 1 mM EGTA and 1% protease inhibitor cocktail, Sigma).

2.2. Primary neuronal cell culture and rotenone exposure

Cell culture method was described in detail elsewhere (Kivell et al., 2001). Briefly, for each experimental group, 20 neonatal (1 day-old) Lewis rats were decapitated and their hippocampus was dissected out, dissociated in sterile cold solution consisted of 120 mM NaCl, 5 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, 13 mM glucose, pH 7.2, and subjected to mechanical and chemical cell decoupling. Solution containing cells was centrifuged at 300 g for 5 min. Supernatant was discarded and cells were suspended in Neurobasal A medium (Gibco) supplemented with 0.25 mM Glutamax (Gibco), 2% B27 (Gibco); 0.25 mM L-Glutamine (Sigma) and 40 mg/L Gentamicin (Gibco).

Cells were plated on 24-well plate (Nunc) or confocal dishes, coated with poli-D-lysine, at the concentration of 1800 cells/mm^2. Cultures were kept in a humidified incubator with 5% CO_2 at 37 °C for nine days with medium changed every three days of cultivation.

Rotenone was prepared with DMSO and diluted in culture medium applied to cell cultures in concentrations of 0.3, 0.5 and 1.0 nM for 48 h, control cultures were exposed to DMSO diluted in culture medium, all cells were exposed to at most 0.001% DMSO.

2.3. Western blot

Brain tissues were homogenized in extraction buffer and centrifuged at 14000 rpm for 20 min; the resulting supernatant was fractionated by SDS-PAGE (15 μg of protein/lane) using a 12% tris-HCl gel at 100 V for 1 h. Proteins were transferred to nitrocellulose membrane in transfer buffer (25 mM Tris, 190 mM glycine, 10% methanol) for 1 h at 100 V at 4 °C. Membranes were blocked for 1 h at room temperature in Tris-Buffered saline containing Tween 20 (TBS-T; 50 mM Tris, pH 8.0, 133 mM NaCl, 0,2% Tween 20) with 5% non-fat dry milk or 3% BSA (Sigma).

Blots were incubated with primary antibodies against BDNF (N-20, sc-546, Santa Cruz, 1/500); TrkB (H-181, sc-8316, Santa Cruz, 1/1000); SLP-1 (sc-136480, Santa Cruz, 1/2000); CRMP-2 (C2993, Sigma, 1/7000); Rab-27B (R-4655, Sigma, 1/1000); EDH-4 (NBP1-54873, Novus, 1/1000); KIF1B (L-20, sc-18739, Santa Cruz, 1/200); Dynein (R-325, sc-9115, Santa Cruz, 1/200); Dynactin (H-300, sc-11363, Santa Cruz, 1/400); AKT (9272, Cell Signaling, 1/2000); pAKT (ser-473, Cell Signaling, 1/750), NeuN (MAB377, Millipore, 1/500), JNK (9258, Cell Signaling, 1/1000) or p53 (2524, Cell Signaling, 1/500), in 3% non-fat dry milk or 1% BSA in TBS-T, overnight at 4 °C, followed by horseradish peroxidase-conjugated anti-mouse (1/6000, Amersham), anti-goat (1/5000, Amersham) or anti-rabbit (1/10000, Amersham). Secondary antibodies incubations were performed at room temperature during 1 h.