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Research Paper

BDNF over-expression induces striatal serotonin fiber sprouting and increases the susceptibility to L-DOPA-induced dyskinesia in 6-OHDA-lesioned rats

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ABSTRACT

In addition to its role in neuronal survival, the brain neurotrophic factor (BDNF) has been shown to influence serotonin transmission and synaptic plasticity, events strongly implicated in the appearance of L-DOPA-induced dyskinesia (LID), a motor complication occurring in parkinsonian patients after long-term treatment with the dopamine precursor.

In order to evaluate a possible influence of BDNF in the appearance of LID, 6-OHDA-lesioned rats received a striatal injection of different concentrations of an adeno-associated viral (AAV) vector over-expressing either BDNF or GFP, as control vector. Eight weeks later, animals started to receive a daily treatment with L-DOPA (4–6 mg/kg plus benserazide 4–6 mg/kg, s.c.) or saline, and dyskinesias, as well as L-DOPA-induced rotations, were evaluated at several time-points. Moreover, molecular changes in striatal D1 receptor-dependent cAMP/PKA and ERK/mTORC signaling pathways, as well as, sprouting of striatal serotonin axons, were measured. Results showed that the AAV-BDNF vector injection induced striatal over-expression of BDNF, as well as striatal and pallidal serotonin axon hyperinnervation. Moreover, rats that over-expressed BDNF were more prone to develop LID and L-DOPA-induced rotations, compared to the GFP-treated control group. Finally, rats that over-expressed BDNF showed increased levels of striatal D1R-dependent signaling phospho-proteins in response to L-DOPA administration. This study suggests that BDNF over-expression, by inducing changes in pre-synaptic serotonin axonal trophism, is able to exacerbate maladaptive responses to L-DOPA administration.

1. Introduction

L-DOPA-induced dyskinesia (LID) is a troublesome side effect that develops in the vast majority of parkinsonian patients under chronic L-DOPA treatment. In recent years, the serotonin system has emerged as a key player in the appearance of LIDs and became a possible target for pharmacological therapies (Bastide et al., 2015; Carta et al., 2007). Indeed, an increasing body of experimental evidence suggests that serotonin neurons can convert L-DOPA into dopamine (DA) and mediate its release as a "false" transmitter. Due to the lack of pre-synaptic mechanisms able to fine-tune L-DOPA-derived DA release, serotonin neurons would contribute to swings in extracellular DA levels after oral administration of L-DOPA, causing, in turn, a pulsatile stimulation of striatal DA receptors, a key event in the appearance of abnormal movements (Arai et al., 1995; Carta et al., 2007; Carta and Tronci, 2014; Iderberg et al., 2015; Tanaka et al., 1999). In agreement with this scenario, toxin lesion or pharmacological blockade of serotonin neuronal activity lead to almost full suppression of LID in experimental

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animal models of Parkinson's disease (PD) (Beaudoin-Gobert et al., 2015; Carta et al., 2007; Muñoz et al., 2008). In line with this evidence, it has been recently demonstrated that LIDs are correlated with the state of the serotonin innervation at striatal level in the rat experimental model of PD as well as in parkinsonian patients; in fact, sprouting of serotonin neuron terminals can further exacerbate development of LID in parkinsonian rats (Rylander et al., 2010).

Rylander et al. have shown that LID correlates with striatal levels of brain-derived neurotrophic factor (BDNF) in 6-OHDA-lesioned rats; this result is in agreement with several studies, which reported that behavioral sensitization to L-DOPA in 6-OHDA-lesioned rats was associated to an upregulation of mRNA levels for BDNF and TrkB receptor in the frontal cortex and striatum (Bordet et al., 1997; Guillin et al., 2003, 2001). Furthermore, several studies suggested that BDNF can produce local trophic effect on serotonergic neurons in the adult intact rat brain, when directly injected as protein (Goggi et al., 2002; Mamounas et al., 2000).

Based on the above evidence, we speculated that striatal injection of an adeno-associated viral (AAV) vector coding for the rat BDNF gene in 6-OHDA-lesioned rats, by over-expressing the neurotrophin, would induce sprouting of serotonin axons, in turn, promoting maladaptive responses to L-DOPA administration and worsening of LID. Here, sprouting of serotonin axons was measured in terms of SERT immunoreactivity, which is considered a reliable index of increased density of 5-HT-positive fibers or axonal varicosities (Bez et al., 2016; Descarries et al., 1995; Rylander et al., 2010). Moreover, based on the existence of a correlation between LID and D1 receptor-dependent cAMP/PKA and ERK/mTORC signaling pathways (Brugnoli et al., 2016; Decressac and Björklund, 2013; Santini et al., 2009b, 2007; Subramaniam et al., 2011), we performed biochemical analysis in order to clarify the molecular features underlying the behavioral effects observed in this study.

2. Material and methods

2.1. Production of the recombinant AAV viral vector

Transfer plasmids carrying adeno-associated viral (AAV5) inverted terminal repeat coding for either rat BDNF or enhanced green fluorescent protein (GFP), downstream of a cytomegalovirus enhancer hybrid synthetic chicken β-actin (CBA) promoter, were generated. Transfection into HEK 293 cells was carried out using the calcium phosphate method, and included the appropriate transfer plasmid encoding-enhanced BDNF or GFP and the packaging plasmids pDP5 encoding for the AAV5 capsid proteins. Cells were transfected with 2.5 mg of DNA with equimolar amounts of helper and transfer DNA. Transfected cells were incubated for 3 days before being harvested in phosphate buffered saline–EDTA. The cell pellet was treated with a lysis buffer (50 mM Tris, 150 mM NaCl, pH 8.4) and lysed through freeze–thaw cycles in a dry ice/ethanol bath. The lysate was then treated with 21 U/ml benzonase (Sigma) for nuclear digestion. The crude lysates were purified first by ultracentrifugation (1.5 h at 350,000*g* at 18 °C) in a discontinuous iodixanol gradient and the virus-containing fractions were purified with ion-exchange chromatography using fast protein liquid chromatography. The virus suspension was then concentrated using a concentrator (Millipore Amicon Ultra, 100 kDa molecular weight cut-off) at 1500 g and 18 °C in two consecutive steps by adding phosphate buffered saline. The physical titres of recombinant AAV5 vectors were determined using dot blot quantification as described previously (Zolotukhin et al., 1999). Genome copy titres were determined using real-time quantitative polymerase chain reaction PCR, and the following vector concentrations were used: 1.2×10^{14} and 3.1×10^{13} genome copies/ml for BDNF and GFP, respectively. The AAV-GFP vector was injected as a control at a dilution that matched the number of genome copy per milliliter of the transgene of interest.

2.2. Animals

Adult male Sprague–Dawley rats (275–300 g; Harlan, Italy) were used in the present study and housed on a 12 h light/dark cycle (light on 7:00–19:00) with free access to food and water. All animal work was performed in accordance with regulations set by the European Union (EEC Council 86/609; D.P.R. 116/92).

2.3. Surgical procedures

2.3.1. Experimental parkinsonism

All 6-OHDA injections were conducted using a stereotaxic frame with an attached Hamilton syringe and under general anesthesia, induced by an i.p. injection of a 20:1 mixture of Fentanest (Pfizer, Italy) and Dormitor® (Orion pharma, Italy) at a volume range of 1.4–1.6 ml. The animals received 6-OHDA injection into the middle forebrain bundle (16 μg free base in 4 μl in 0.02% L-ascorbic acid in 0.9% saline), at the following coordinates relative to the bregma (Paxinos and Watson, 2007): AP: − 4.4 mm, ML: − 1.2 mm; DV: − 7.8 mm from the dura, in order to achieve a complete lesion of the nigrostriatal pathway. Injection speed was 1.0 μl/min and the syringe was kept in place for an additional 3 min period before it was slowly retracted.

2.3.2. AAV vector injection

Surgical procedure for AAV vector injection was performed under general anesthesia, as used for 6-OHDA. To give less damage to the brain, a glass capillary (outer diameter 60–80 μm) was fitted onto the needle of a 5 μl Hamilton syringe. Rats received 4 μl (2 μl/site; 1 μl/deposit) of the AAV-BDNF or AAV-GFP solution into the 6-OHDA-lesioned striatum at the following coordinates (flat skull position) relative to the bregma (Paxinos and Watson, 2007): AP/ML = + 1/− 2.8 mm, DV = − 5/− 4 mm; AP/ML = + 0.2/− 4 mm, DV = − 5.5/− 4.5 mm from the dura surface. Injection speed was 0.5 μl/min and the needle was left in place for an additional 3 min period before it was slowly retracted.

2.4. Experimental design

2.4.1. Effect of BDNF over-expression on LID

In order to have a complete picture of the effect of BDNF over-expression on development of dyskinesia, we performed different consecutive experiments with escalating dilutions of the vector. Thus, we started with an undiluted vector and then we progressively lowered the concentration, up to 3% of the initial concentration. This was done because it has been demonstrated that, depending on the dose, neurotrophic factors may have a dual action on specific brain functions (Arias et al., 2014; Mamounas et al., 2000; Paredes et al., 2007). Thus, in a first set of experiments (Exp. 1, Fig. 1), three weeks after the 6-OHDA lesion ($n = 20$), rats were subjected to the stepping test in order to include in the study only rats with a severe impairment (stepping test score between 0 and 2, data not shown). One week later, rats having a mean not higher than two steps were divided into two well-matched groups and subjected to the striatal injection of an undiluted AAV vector solution for the expression of BDNF, or GFP (as a control). Eight weeks after, rats that over-expressed BDNF or GFP were chronically treated with L-DOPA (6 mg/kg plus benserazide 6 mg/kg, s.c., $n = 8$/group), and abnormal involuntary movements (AIMs) were scored for 3 weeks. L-DOPA-induced rotational behavioral was also evaluated at day 7 and 15 of L-DOPA treatment. At the end of the behavioral tests, rats were killed and the brain processed for the tyrosine hydroxylase (TH) immunohistochemical evaluation.

One more group of 6-OHDA-lesioned rats (Exp. 2, Fig. 1) were injected with different AAV vector dilutions for BDNF (10% and 20%) or GFP, and LID was evaluated eight weeks after the virus injection. For this experiment we also used a lower dose of L-DOPA (4 mg/kg plus benserazide 4 mg/kg, s.c.) to better highlight the difference in AIM