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Combination of CDNF and Deep Brain Stimulation Decreases Neurological Deficits in Late-stage Model Parkinson's Disease

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Abstract—Several neurotrophic factors (NTF) are shown to be neuroprotective and neurorestorative in pre-clinical animal models for Parkinson's disease (PD), particularly in models where striatal dopamine neuron innervation partially exists. The results of clinical trials on late-stage patients have been modest. Subthalamic deep brain stimulation (STN DBS) is a proven treatment for a selected group of advanced PD patients. The cerebral dopamine neurotrophic factor (CDNF) is a promising therapeutic protein, but its effects in animal models of late-stage PD have remained under-researched. The interactions of NTF and STN DBS treatments have not been studied before. We found that a nigral CDNF protein alone had only a marginal effect on the behavioral deficits in a late-stage hemiparkinsonian rat model (6-OHDA MFB). However, CDNF improved the effect of acute STN DBS on front limb use asymmetry at 2 and 3 weeks after CDNF injection. STN lesion—modeling chronic stimulation—had an additive effect in reducing front limb use in the cylinder test and apomorphine-induced rotation. The combination of CDNF and acute STN DBS had a favorable effect on striatal tyrosine hydroxylase. This study presents a novel additive beneficial effect of NTF and STN DBS, which might be explained by the interaction of DBS-induced endogenous NTFs and exogenously injected CDNF. SNpc can be reached via similar trajectories used in clinical STN DBS, and this interaction is an important area for future studies.

Key words: CDNF, MANF, GDNF, neurotrophic factor, median forebrain bundle, 6-hydroxydopamine, STN DBS.

INTRODUCTION

Parkinson's disease (PD) is a progressive neurodegenerative disease. The cardinal symptoms of PD—tremor, rigidity, postural instability, and bradykinesia—are mainly caused by shortage of dopamine due to dopamine neuron death in the substantia nigra pars compacta (SNpc). The best available drugs—levodopa in combination with DDC and COMT inhibitors and dopamine agonists—act by replacing lost dopamine in the brain, leading to alleviation of the motor symptoms. However, in the late stages of the disease, these drugs lose their effectiveness or begin to cause debilitating side effects. Deep brain stimulation (DBS) of the subthalamic nucleus (STN) provides an efficient symptomatic control for some patients whose PD has progressed to a late stage (Krack et al., 2003; Castrioto et al., 2011).

Experimental DBS is often termed high-frequency stimulation (HFS), alluding to the finding that only frequencies higher than about 60 Hz alleviate the motor symptoms produced in the animal models of PD (Fogelson et al., 2005), and the standard 130-Hz frequency used produces mainly the inhibition of STN cells (Tai et al., 2003). In most animal studies, stimulation comes from an external pulse generator that limits the duration of continuous HFS to hours, or at the most, days. Long-term STN HFS and DBS can be mimicked by the lesioning of the STN (STNL), and originally DBS was realized to reproduce the effects of therapeutic stereotactic lesions (Benazzouz et al., 1993). The efficacy of STNL was originally verified by chemical ibotenic acid (IBOT) lesions in a primate MPTP model of PD (Bergman

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Abbreviations: 5-HT, serotonin, 6-OHDA, 6-hydroxydopamine; CDNF, cerebral dopamine neurotrophic factor; DA, dopamine; DBS, deep brain stimulation; DOPAC, 3,4-dihydroxyphenylacetic acid; GDNF, glial cell line-derived neurotrophic factor; HVA, homovanillic acid; IBOT, ibotenic acid; MANF, mesencephalic astrocyte-derived neurotrophic factor; MFB, medial forebrain bundle; NTF, neurotrophic factor; PD, Parkinson's disease; SNpc, substantia nigra pars compacta; STN, subthalamic nucleus; STNL, lesion of subthalamic nucleus; TH, tyrosine hydroxylase.

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et al., 1990). Furthermore, STNL has been used in patients with PD (Jourdain et al., 2014). Additionally, STN DBS has both local excitatory effects aside from inhibition (McIntyre et al., 2004) and complex network level effects (McIntyre and Hahn, 2010) such as effects on beta coupled high-frequency activity in motor cortex (Yang et al., 2014). According to animal studies, DBS is able to cause increased striatal dopamine transmission (Meissner et al., 2001; Walker et al., 2009; Pazo et al., 2010) and affect the downstream motor nuclei (Shehab et al., 2014). There is some experimental evidence that DBS may have a neuroprotective effect (Temel et al., 2006; Harnack et al., 2008; Spieles-Engemann et al., 2010; Musacchio et al., 2017), but clinical evidence for neuroprotection is lacking (Harnack and Kupsch, 2010).

There has been an extensive search for drugs that can stop the progression of dopamine neuron degeneration and restore dopaminergic phenotype and function in dying neurons (Airavaara et al., 2012b; Bartus and Johnson, 2017a,b). Neurotrophic factors (NTFs) are able to protect and even restore dopamine neuron degeneration. NTFs are a group of proteins with variable biological actions (Airaksinen and Saarma, 2002; Lindholm et al., 2016). Importantly, both glial cell line-derived neurotrophic factor (GDNF) and neurturin (NRTN) have been shown to be neurorestorative in rodent and primate models of PD *in vivo* (Airavaara et al., 2012b). The efficacy has been found in partial lesion models where tyrosine hydroxylase (TH) immunoreactive fibers in the striatum still exist. Recently, it was reported that the retrograde transport of cerebral dopamine neurotrophic factor (CDNF) from the striatum to substantia nigra (Voutilainen et al., 2011) depends on striatal dopamine innervation (Mätlik et al., 2017). Additionally, BDNF (Spina et al., 1992), FGF-2 (Otto and Unsicker, 1990) and VEGF (Yasuhara et al., 2004) have been effective preclinical trials. Two NTFs—GDNF and neurturin—have been in clinical trials, where they have been administered directly into the striatum either GDNF as a recombinant protein or NRTN in an adeno-associated virus (AAV) gene therapy, but their efficacy has been variable (Kordower et al., 1999; Gill et al., 2003; Lang and Obeso, 2004; Lang et al., 2006; Penn et al., 2006; Patel and Gill, 2007; Bartus and Johnson, 2017b). Clinical studies have usually been carried out on late-stage patients, but a recent NRTN study showed positive effects in a subgroup of patients with less-progressed disease (Olanow et al., 2015). Analysis of the brains of PD patients has shown that there is minimal or no putaminal TH immunoreactive innervation after 5 years post-diagnosis (Kordower et al., 2013). Therefore, the relatively rapid development of putaminal dopaminergic axonopathy should be taken into account in future trials (Tenenbaum and Humbert-Claude, 2017), particularly when there are radiolabeled ligands with which to study the functionality of dopamine terminals. Similarly, several NTFs have been effective in partial lesion models of PD; but with complete lesions, they have not restored dopamine neurites in the striatum (Airavaara et al., 2012b; Domanskyi et al., 2015).

The CDNF/MANF protein family has a neurorestorative potential similar to GDNF and NRTN (Lindholm et al., 2007; Voutilainen et al., 2009; Lindahl et al., 2017). In a rat 6-OHDA model of PD where 6-OHDA was administered to the striatum, both CDNF and mesencephalic astrocyte-derived neurotrophic factor (MANF) have been shown to be neuroprotective and neurorestorative (Lindholm et al., 2007; Voutilainen et al., 2009, 2011; Ren et al., 2013; Bäck et al., 2013a). This was also the case for CDNF in a mouse MPTP model of PD (Airavaara et al., 2012a). However, the effects of CDNF have not been studied in medial forebrain (MFB) 6-OHDA models of PD where dopamine depletion is nearly complete. In the clinic, STN DBS is used mostly for late-stage PD, where the caudate putamen is devoid of TH+ fibers and dopamine depletion is already severe. Therefore, the likelihood of any neurorestorative therapy alone increasing endogenous dopaminergic activity is limited.

The aim of the current experiments was to study whether CDNF has a neurorestorative effect in a late-stage parkinsonian model (MFB lesion) and whether CDNF and DBS are synergistically neurorestorative in this model. A MFB 6-OHDA lesion was used instead of a striatal lesion, and CDNF in combination with or without STN DBS was employed. STNL was used to model long-term DBS.

EXPERIMENTAL PROCEDURES

Animals

A total of 242 male Wistar rats weighing 220–300 g at the time of first operation were used. Rats were housed under a light–dark cycle at an ambient temperature of 20–23 °C. Food pellets (Harlan Teklad Global diet, Holland) and tap water were available *ad libitum*. The experimental design was approved by the Committee for Animal Experiments of the University of Helsinki and the chief veterinarian of the County Administrative Board for 2008–2010 and by the National Animal Experiment Board for 2011–2015. Animal experiments were conducted according to EU regulations (EU Directive 2010/63/EU) and Finnish legislation (Finnish Act on the Protection of Animals Used for Scientific or Educational Purposes [497/2013] and the Government Decree on the Protection of Animals Used for Scientific or Educational Purposes). The laboratory experiment protocol approval numbers were ESAVI/5459/04.10.03/2011 and ESAVI/6959/04.10.03/2012.

Surgical procedures

The stereotaxic operations were performed under isoflurane anesthesia, as described in previous studies (Lindholm et al., 2007); (Voutilainen et al., 2009). 6-Hydroxypamine (6-OHDA, 10 μg) was injected into either the left anterior (A/P −2.0, L/M +2.0, D/V −8.3; adapted from (Shi et al., 2004) for the STN HFS and STN lesioning experiments (Experiments 3 and 4) or left posterior medial forebrain bundle (A/P −4.4, L/M +1.2, D/V −8.3) as described in (Hudson et al., 1993, 1994) for the NTF only experiments (Experiments 1 and 2). NTFs (GDNF, Amgen, Thousand Oaks, CA, USA; human recombinant

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