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Tricyclic antidepressant treatment evokes regional changes in neurotrophic factors over time within the intact and degenerating nigrostriatal system

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ABSTRACT

In addition to alleviating depression, trophic responses produced by antidepressants may regulate neural plasticity in the diseased brain, which not only provides symptomatic benefit but also potentially slows the rate of disease progression in Parkinson's disease (PD). Recent *in vitro* and *in vivo* data provide evidence that neurotrophic factors such as brain-derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF) may be key mediators of the therapeutic response to antidepressants. As such, we conducted a cross-sectional time-course study to determine whether antidepressant-mediated changes in neurotrophic factors occur in relevant brain regions in response to amitriptyline (AMI) treatment before and after intrastriatal 6-hydroxydopamine (6OHDA). Adult male Wistar rats were divided into seven cohorts and given daily injections (i.p.) of AMI (5 mg/kg) or saline throughout the duration of the study. In parallel, various cohorts of intact or parkinsonian animals were sacrificed at specific time points to determine the impact of AMI treatment on trophic factor levels in the intact and degenerating nigrostriatal system. The left and right hemispheres of the substantia nigra, striatum, frontal cortex, piriform cortex, hippocampus, and anterior cingulate cortex were dissected, and BDNF and GDNF levels were measured with ELISA. Results show that chronic AMI treatment elicits effects in multiple brain regions and differentially regulates levels of BDNF and GDNF depending on the region. Additionally, AMI halts the progressive degeneration of dopamine (DA) neurons elicited by an intrastriatal 6-OHDA lesion. Taken together, these results suggest that AMI treatment elicits significant trophic changes important to DA neuron survival within both the intact and degenerating nigrostriatal system.

Introduction

Depression is a common co-morbid disorder that affects almost half of all patients with Parkinson's disease (PD), many of which take antidepressants on a daily basis (Ravina et al., 2007). Although a number of studies have investigated the trophic response to various antidepressant drugs (Martinez-Turrillas et al., 2005; Xu et al., 2003; Nibuya et al., 1995, 1996, 1999; Hisaoka et al., 2001, 2007; Mercier et al., 2004; Rogoz and Legutko, 2005; Coppell et al., 2003), most focus on changes within mesolimbic brain structures. Therefore, little is known about whether antidepressant therapy impacts trophic factors within the nigrostriatal dopamine (DA) system. The trophic factors brain-derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF) are of particular importance for nigrostriatal DA neurons vulnerable to degeneration in PD (Allen et al., 2013). While both factors have shown promise for PD pre-clinically (Sauer et al., 1994; Zhou et al., 1997; Olson, 1990; Yurek et al., 1996; Altar et al., 1992; Bjorklund et al., 1997; Zawada et al., 1998; Kordower et al., 2000; Mendez et al., 2000; Hebb et al., 2002; McBride and Kordower, 2002; Kirik et al., 2004), only GDNF has been investigated for utility in human trials. Multiple open-label clinical trials demonstrated significant symptomatic improvement in PD patients that received intraputamenal GDNF infusion (Patel et al., 2005; Slevin et al., 2005; Gill et al., 2003). However, a randomized controlled trial of intraputamenal GDNF infusion yielded negative results (Lang et al., 2006), thereby dampening the enthusiasm for exogenous delivery of this protein in the clinic. Although unexpected, this finding does not negate the positive data supporting the neuroprotective potential of trophic factors; it merely indicates that *exogenous* delivery of trophic support may not be an optimal strategy, especially in end stage PD. Therefore, treatments that induce the synthesis or release of *endogenous* factors in the brain, particularly

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earlier in the disease process, may provide a more effective strategy for treating PD patients (Castren, 2004a,b).

Psychopharmacological agents such as antidepressants have been shown to modulate important signaling pathways involved in cell survival and plasticity (Xu et al., 2003; Nibuya et al., 1995, 1996), thereby suggesting they may have more pleiotrophic ability than previously anticipated. Such studies provide evidence that antidepressant treatment could evoke changes that induce neuroprotection in neurodegenerative diseases, including PD. The trophic response elicited by chronic antidepressant treatment has been well documented within mesolimbic regions involved in depression and mood disorders; however, little is known about whether similar changes occur in the nigrostriatal pathway and hence may provide dual therapeutic value for PD patients. Additionally, there are conflicting data concerning the onset and duration of antidepressant-mediated trophic responses (De Foubert et al., 2004; Khundakar and Zetterstrom, 2006). Therefore, we performed a cross-sectional time-course study to determine the effects of chronic antidepressant treatment on BDNF and GDNF levels in brain regions relevant to PD. As tricyclic antidepressants (TCAs) have been shown to be more efficacious in treating depression in PD patients (Chung et al., 2010; Menza et al., 2009), we opted to examine the specific TCA amitriptyline (AMI), which is often prescribed to patients with PD (Chen et al., 2007). Furthermore, we recently reported that AMI, but not other antidepressants, was associated with a delay in the need to start dopaminergic therapy in an early cohort of patients with PD (Paumier et al., 2012), suggesting it may have disease-modifying properties. More recently, our laboratory demonstrated that the low dose of AMI used in this study afforded significant neuroprotection to the nigrostriatal system in a rat toxin model of parkinsonism (Paumier et al., 2014). We report here that chronic AMI treatment differentially regulates BDNF and GDNF in the intact and degenerating nigrostriatal system, and that AMI significantly retards the progression of nigral dopamine neuron loss.

Methods

Animals. Animals were housed and treated following the National Institutes of Health guidelines (Institute of Laboratory Animal Research C.o.L.S., 1996). Each experiment utilized adult (4- to 6-month-old) male Wistar rats (200–225 g) as subjects. Rats were obtained from Harlan Animal Research Laboratory (Indianapolis, IN). The animal facility at the University of Cincinnati is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care and complied with all Federal animal care and use guidelines. The Institutional Animal Care and Use Committee (IACUC) at the University of Cincinnati, where these studies were performed, approved all protocols pertaining to these experiments.

Experimental design. Rats were acclimated to their new environment for 24 h before beginning experiments then randomized and divided into four groups (saline—no lesion, amitriptyline (AMI)—no lesion, saline + lesion, and AMI + lesion) and given daily intraperitoneal (i.p.) injections of sterile saline (Henry Schein) or 5 mg/kg of amitriptyline hydrochloride (AMI, Sigma) for 14 days (Fig. 1A). This 14-day pretreatment regimen and low dosage was based on previous studies indicating that this timing is important to achieve antidepressant efficacy (Xu et al., 2003; Nibuya et al., 1996; Coppell et al., 2003). On day 14, rats in the appropriate groups (+lesion) received unilateral intrastriatal injection of the DA neurotoxin 6-hydroxydopamine (6-OHDA), after which all rats continued to receive daily i.p. AMI or saline injections for the remainder of the experiment (4 weeks). Lesioned and non-lesioned cohorts of rats from both saline and AMI groups were sacrificed on days 14, 17, 24, and 42, which corresponds to the following days post-lesion: day 0 (D0), day 3 (D3), day 10 (D10), and day 30 (D30). All rats were sacrificed at prescribed time points, and brains were extracted and processed for either immunohistochemical or ELISA analyses.

To assess trophic factor levels within the nigrostriatal system, we examined BDNF and GDNF protein in the SN and striatal structures. However, BDNF is not produced in the striatum (Altar and DiStefano, 1998; Altar et al., 1997) yet receives BDNF protein via anterograde/retrograde transport from various interconnected regions including the SN (Mendez and Hong, 1997), hippocampus (Gasbarri et al., 1994, 1996), frontal (Altar and DiStefano, 1998; Altar et al., 1997; Mufson et al., 1999), piriform (Rite et al., 2005; Datiche and Cattarelli, 1996), and anterior cingulate cortices (Shibata and Naito, 2008; Calzavara et al., 2007; Domesick, 1969; Takada and Hattori, 1986) (Fig. 1B). Therefore, we extended the scope of the project to include these structures, and each hemisphere was microdissected and processed as an independent sample as described previously (Paumier et al., 2014).

6-OHDA lesions. Prior to surgery, all rats were anesthetized (30 mg/kg, pentobarbital, i.p.) and placed into a stereotaxic device. The 6-OHDA (dissolved in a 0.2% ascorbic acid-physiological saline) was made immediately prior to surgery and kept on ice. All rats received 2 μl of 6-OHDA (5 μg/μl) into the ipsilateral left striatum (AP +0.7, ML +3.0, DV −5.5 from skull) at a rate of 0.5 μl per minute. After each injection, the needle was left in place for 2 min and then slowly withdrawn. Lesions were verified by stereological assessment of tyrosine hydroxylase immunoreactive (THir) neurons within the substantia nigra pars compacta (SNpc).

Immunohistochemistry. Animals were euthanized via pentobarbital overdose (60 mg/kg) and intracardially perfused with 0.9% saline followed by ice-cold 4% paraformaldehyde (PFA) in 0.1 M PO^4 buffer. Brains were extracted and post-fixed in 4% PFA for 24 h and sunk in 30% sucrose. Brains were cut into 40 μm thick sections on a freezing microtome. A 1:6 series of coronal sections was double labeled for tyrosine hydroxylase (TH) (marker of dopamine neurons) and NeuN (general neuronal marker) using the free-floating method. A sequential approach was utilized to visualize these proteins using antibodies from the same host (mouse) (van der Loos, 2008). Tissue was incubated in 0.3% H_20_2 for 45 min, rinsed and blocked in 10% normal goat serum (1 h), then incubated in primary mouse anti-NeuN antibody (1:500; Chemicon) overnight at 4 °C. Following primary incubation, sections were incubated in biotinylated secondary antisera against mouse IgG (1:400, Chemicon) followed by the Vector ABC detection kit employing horseradish-peroxidase (Vector Labs). Antibody labeling was visualized by exposure to 0.5 mg/ml 3,3′ diaminobenzidine (DAB), 2.5 mg/ml nickel ammonium sulfate, and 0.03% H_2O_2 in Tris buffer. Tissue was rinsed and blocked again overnight at 4 °C. The following day, tissue was incubated in mouse anti-TH antibody (1:4000, Chemicon) for 4 h, followed by biotinylated secondary antisera against mouse IgG (same as above) and the Vector ABC detection kit. Labeling was visualized using the NovaRed kit (Vector Labs), and sections were mounted on subbed slides, dehydrated to xylene and coverslipped with Cytoseal (Richard-Allan Scientific). Since TH is localized to the cytoplasm, any cross reactivity to the biotinylated secondary antisera against mouse IgG labeling did not interfere with nuclear NeuN visualization.

ELISA. Animals were anesthetized (60 mg/kg, pentobarbital, i.p.) and perfused with heparinized saline (0.9%). Brains were flash frozen in 3-methyl butane (Fisher) then stored at −80 °C. 1–2 mm coronal slabs were blocked utilizing a brain blocker (Zivic, Pittsburg, PA), and both hemispheres of the SN, striatum, hippocampus, frontal cortex, anterior cingulate cortex (ACC) and the piriform cortex were microdissected at a constant −12 °C on a cold plate (Teca, Chicago, IL) and stored at −80 °C until analysis. Samples were homogenized on ice in 200 μl of lysis buffer (M-PER; Pierce) with protease inhibitors (Sigma-Aldrich). The Pierce BCA Protein Kit (Rockford, IL) was utilized for protein determination. The homogenate was centrifuged at 4 °C for 20 min at 14,000g, and the supernatant was collected. ELISAs were run using the Immunolon 4 HBX 96-well plate according to manufacturer's instructions (GDNF/BDNF Promega Emax ImmunoAssay Kit). Samples