Pharmacology, Biochemistry and Behavior xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Glial cell-line derived neurotrophic factor (GDNF) replacement attenuates motor impairments and nigrostriatal dopamine deficits in 12-month-old mice with a partial deletion of *GDNF*

Ofelia M. Littrell [a], Ann-Charlotte Granholm [b], Greg A. Gerhardt [a], Heather A. Boger [b,*]

[a] Department of Anatomy and Neurobiology, Parkinson's Disease Translational Research Center of Excellence, University of Kentucky Medical Center, 306 Davis Mills Bldg., 800 Rose St., Lexington, KY 40536, USA
[b] Department of Neurosciences and Center on Aging, Medical University of South Carolina, 173 Ashley Ave., BSB Suite 403, Charleston, SC 29425, USA

ARTICLE INFO

Article history:
Received 4 September 2012
Received in revised form 5 December 2012
Accepted 22 December 2012
Available online xxxx

Keywords:
Dopamine
Glial cell-line derived neurotrophic factor
Parkinson's disease
Neurodegeneration
Striatum
Substantia nigra

ABSTRACT

Glial cell-line derived neurotrophic factor (GDNF) has been established as a growth factor for the survival and maintenance of dopamine (DA) neurons. In phase I clinical trials, GDNF treatment in Parkinson's disease patients led to improved motor function and asGDNF has been found to be down regulated in Parkinson's disease patients. Studies using GDNF heterozygous (*Gdnf*$^{+/-}$) mice have demonstrated that a partial reduction of GDNF leads to an age-related accelerated decline in nigrostriatal DA system- and motor-function and increased neuro-inflammation and oxidative stress in the substantia nigra (SN). Therefore, the purpose of the current studies was to determine if GDNF replacement restores motor function and functional markers within the nigrostriatal DA system in middle-aged *Gdnf*$^{+/-}$ mice. At 11 months of age, male *Gdnf*$^{+/-}$ and wildtype (WT) mice underwent bilateral intra-striatal injections of GDNF (10 μg) or vehicle. Locomotor activity was assessed weekly 1–4 weeks after treatment. Four weeks after treatment, their brains were processed for analysis of GDNF levels and various DAergic and oxidative stress markers. An intrastriatal injection of GDNF increased motor activity in *Gdnf*$^{+/-}$ mice to levels comparable to WT mice (1 week after injection) and this effect was maintained through the 4-week time point. This increase in locomotion was accompanied by a 40% increase in striatal GDNF protein levels and SN GDNF expression in *Gdnf*$^{+/-}$ mice. Additionally, GDNF treatment significantly increased the number of tyrosine hydroxylase (TH)-positive neurons in the SN of middle-aged *Gdnf*$^{+/-}$ mice, but not WT mice, which was coupled with reduced oxidative stress in the SN. These studies further support that long-term changes related to the dysfunction of the nigrostriatal pathway are influenced by GDNF expression and add that this dysfunction appears to be responsive to GDNF treatment. Additionally, these studies suggest that long-term GDNF depletion alters the biological and behavioral responses to GDNF treatment.

1. Introduction

The use of neurotrophic factor supplementation, particularly in the context of Parkinson's disease (PD), has been widely investigated with promising findings of enhanced neuron function and behavioral measures (Peterson and Nutt, 2008). In particular, GDNF has shown restorative effects in numerous animal models exhibiting dopamine (DA)-neuron dysfunction including the aged and 6-hydroxydopamine (6-OHDA) lesioned rat (Hebert and Gerhardt, 1997; Hoffer et al., 1994) and the aged and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned non-human primate (Grondin et al., 2002, 2003). GDNF has also demonstrated protective effects from 6-OHDA- and MPTP-induced cytotoxicity in rodents (Kearns et al., 1997; Tomac et al., 1995). Furthermore, in phase I clinical trials GDNF showed significant therapeutic potential in Parkinson's disease patients (Gill et al., 2003; Slevin et al., 2005) with a 25% improvement on the Unified Parkinson Disease Rating Scale motor score.

GDNF is a target derived neurotrophic factor that is expressed at highest levels in the developing striatum with a decline in expression in adulthood (Stromberg et al., 1993). There is also evidence for decreased GDNF expression in the brains of Parkinson's disease patients (Chauhan et al., 2001; Jenner and Olanow, 1998) and dysregulation in aged rats with 6-OHDA lesions (Yurek and Fletcher-Turner, 2001). In light of the restorative- and protective-effects of GDNF on DA neurons and the prominent role of GDNF in development (Granholm et al., 2000), the effects of a chronic GDNF depletion have been investigated using GDNF heterozygous mice (*Gdnf*$^{+/-}$), which have decreased GDNF protein expression in the brain (Boger et al., 2006; Pichel et al., 1996). *Gdnf*$^{+/-}$ mice display a unique aging phenotype — exhibiting

* Corresponding author at: Department of Neurosciences, Medical University of South Carolina, 173 Ashley Ave., BSB Suite 403, Charleston, SC 29425, USA. Tel.: +1 843 876 2230; fax: +1 843 792 4423.
E-mail address: boger@musc.edu (H.A. Boger).

0091-3057/$ – see front matter © 2012 Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.pbb.2012.12.022

locomotor deficiencies, decreases in tyrosine hydroxylase (TH) staining in the SN, and functional changes in DA-release and -uptake in the striatum (Boger et al., 2006; Littrell et al., 2010). Typically, motor and DA-neuron functional measures decline around 12 months of age in *Gdnf*$^{+/-}$ mice.

It has been suggested that inflammation contributes to nigrostriatal dysfunction in *Gdnf*$^{+/-}$ mice because of increased microglial cell activation and exacerbated microglial responses in methamphetamine-induced toxicity models (Boger et al., 2007). Indeed, neuro-inflammation is strongly implicated in the degeneration and dysfunction of the nigrostriatal pathway related to PD and parkinsonism (He et al., 2001; Hunter et al., 2007; Zecca et al., 2008). The inflammatory response is thought to be related to oxidative stress (Hald and Lotharius, 2005) — both processes having been associated with neurodegeneration (Aubin et al., 1998; Chan et al., 2012; Jenner and Olanow, 1996; Sugama et al., 2003). In addition to being linked to PD pathogenesis (Jenner and Olanow, 2006), oxidative stress is implicated in age-associated neurodegeneration (Chakrabarti et al., 2011). Since GDNF treatment reduces neurotoxicity related to oxidative stress (Ortiz-Ortiz et al., 2011; Sawada et al., 2000), oxidative stress markers in *Gdnf*$^{+/-}$ mice were investigated. Preliminary data from our laboratory have shown that markers of oxidative stress are altered in the SN of *Gdnf*$^{+/-}$ mice. Cyclooxygenase-2 (COX-2) is a known cytokine that can be released from glial cells and is involved in neuro-inflammatory and oxidative stress pathways (Gupta et al., 2011).

To further investigate results from preliminary studies, the current studies assess oxidative stress markers (COX-2) as well as levels of an antioxidant (superoxide dismutase-2 (SOD-2)) in this model of GDNF depletion and examine if GDNF treatment in middle-aged *Gdnf*$^{+/-}$ mice affects these oxidative stress markers. Motor behavior (locomotor activity) and DA-neuron functional measures are enhanced in studies using GDNF treatment in animal models (Grondin et al., 2003; Hebert and Gerhardt, 1997; Kordower et al., 2000). Thus, similar locomotor measures and DA-neuron functional measures were investigated after GDNF treatment in *Gdnf*$^{+/-}$ mice. The primary aim of these studies was to test the hypothesis that age-related DA-neuron dysfunction, potential causes of dysfunction, and concomitant motor impairments are reduced by GDNF treatment in *Gdnf*$^{+/-}$ mice. In particular, the following questions were investigated in *Gdnf*$^{+/-}$ and age-matched WT mice: 1) Does GDNF treatment affect spontaneous or stimulated locomotor activity? 2) Does GDNF treatment restore the number of DA neurons in the SN? 3) Does GDNF treatment attenuate oxidative stress markers in the SN?

2. Material and methods

2.1. Animals

A nonfunctional GDNF allele was generated by replacing part of exon 3, which encodes the GDNF protein with a selectable marker neomycin phosphotransferase expressing cassette. Generation and genotyping of *Gdnf*$^{+/-}$ mice is described in detail in previous work (Pichel et al., 1996). Mice were obtained from a colony established at the Medical University of South Carolina. Mice were bred on a C57Bl/6J background consistent with NIH approved protocols. After transfer to the University of Kentucky, mice were acclimated for a minimum of 1 week before experimentation. Male *Gdnf*$^{+/-}$ mice (12 months of age) were compared with age-matched WT mice in all experiments. Mice were housed 3–4 per cage with food and water provided ad libitum. Mice were maintained under 12:12 h light/dark cycle at an ambient temperature of 20–22 °C. Protocols for animal care were in agreement with NIH approved guidelines and compliant with local institutional protocols at the University of Kentucky Medical Center and Medical University of South Carolina. Procedures were in strict agreement with the *Guide for the Care and Use of Laboratory Animals*.

2.2. Reagents

Recombinant methionyl human GDNF (Amgen, Thousand Oaks, CA, USA) expressed in Escherichia coli as previously described (Lin et al., 1993) was used.

2.3. Delivery of GDNF

GDNF was dissolved (5 μg/μL) in sterile (0.22 μm filtered) citrate buffer (10 mM sodium citrate, 150 mM NaCl, pH = 5) as previously described (Hebert et al., 1996).

Animals used for survival surgical procedures were anesthetized with isoflurane gas (1.5–2.5% in O_2) and positioned in a stereotaxic frame (Kopf Instruments, Tujunga, CA, USA). A craniotomy was performed for access to the targeted structures and GDNF or vehicle was delivered using a 26-gauge needle (cannula only) (22026-01, point style-3; Hamilton Company, Reno, NV) attached to a 25-μL Hamilton syringe (80408, point style 3; Hamilton Company, Reno, NV) using plastic tubing (Zeus Inc., Orangeburg, SC). *Gdnf*$^{+/-}$ mice (12-month-old) ($n = 14$) and age-matched WT littermate mice ($n = 8$) were treated with 10 μg of GDNF (bilaterally) or equivalent volume of citrate buffer (vehicle) to the striata. The dose was selected based on previous studies in rodents (Hebert et al., 1996; Hudson et al., 1995). Stereotaxic coordinates were (from *bregma* (mm) (bilaterally)): anterior–posterior: +1.0, medial–lateral: +/−1.5, dorsal–ventral: −3.0 (Franklin and Paxinos, 2001; Kirik et al., 2004). Solution delivery was controlled using a KD Scientific model infusion pump (model 100, KD Scientific Inc., Holliston, MA). Solution delivery began 5 min after lowering to the appropriate depth. GDNF and vehicle treatments were administered bilaterally (10 μg per hemisphere or equivalent volume (2 μL) of vehicle) at a rate of 0.2 μL/min for 10 min. The needle remained in the brain after completing solution delivery and was slowly retracted after 10 min. This procedure was repeated bilaterally and the overlying burr holes were covered with bone wax before closing the incision with dissolvable sutures (4-0 Caprosyn™, Covidien; Norwalk, CT). Topical analgesic ointment (Neosporin® with pramoxine HCl; Rite Aid Corp.) was applied to the incision site following surgical procedures and daily out to 3 days post-operatively. During surgical procedures and the immediate recovery period following surgery, animals rested on a heating pad connected to a re-circulating water bath (Gaymar Industries, Inc., Orchard Park, NY) maintained at 37 °C. Animals recovered in their home cage under observation in the laboratory (~2 h) before transport to the animal housing facility. Animal health was assessed daily for a minimum of 1 week for signs of postoperative distress. There was significant attrition due to anesthetic intolerance in all treatment groups and genotypes. Thus, some of the treated animals were not viable for use in brain tissue analysis. The resulting sample size is indicated in the Results sections.

2.4. Locomotor activity

Locomotor activity (total distance traveled) was assessed using a Digiscan Animal Activity Monitor system (Omnitech Electronics Model RXYZCM (8); TAO, Columbus, OH, USA), details of which have been previously described (Halberda et al., 1997). Animals were tested for spontaneous motor activity prior to treatment with vehicle or GDNF and randomly assigned to treatment groups. Spontaneous motor activity was determined weekly (1–4 weeks after treatment) using the total distance traveled over a 1-hour period. At the 4-week time point, animals were injected with saline (0.9% NaCl, 0.01 mL/g body weight, i.p.) before measuring spontaneous locomotor activity. The saline injection served as a negative control for stimulated motor activity. Previous studies from our laboratory have demonstrated that *Gdnf*$^{+/-}$ mice have greater DAT activity (Boger et al., 2007). Since it has been established in the

Please cite this article as: Littrell OM, et al, Glial cell-line derived neurotrophic factor (GDNF) replacement attenuates motor impairments and nigrostriatal dopamine deficits in 12-month-old mice ..., Pharmacol Biochem Behav (2013), http://dx.doi.org/10.1016/j.pbb.2012.12.022