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Enhanced dopamine transporter activity in middle-aged Gdnf heterozygous mice

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Abstract

Glial cell line-derived neurotrophic factor (GDNF) supports the viability of midbrain dopamine (DA) neurons that degenerate in Parkinson's disease. Middle-aged, 12 month old, *Gdnf* heterozygous (*Gdnf*$^{+/-}$) mice have diminished spontaneous locomotor activity and enhanced synaptosomal DA uptake compared with wild type mice. In this study, dopamine transporter (DAT) function in middle-aged, 12 month old *Gdnf*$^{+/-}$ mice was more thoroughly investigated using *in vivo* electrochemistry. *Gdnf*$^{+/-}$ mice injected with the DAT inhibitor, nomifensine, exhibited significantly more locomotor activity than wild type mice. *In vivo* electrochemistry with carbon fiber microelectrodes demonstrated enhanced clearance of DA in the striatum of *Gdnf*$^{+/-}$ mice, suggesting greater surface expression of DAT than in wild type littermates. Additionally, 12 month old *Gdnf*$^{+/-}$ mice expressed greater D_2 receptor mRNA and protein in the striatum than wild type mice. Neurochemical analyses of striatal tissue samples indicated significant reductions in DA and a faster DA metabolic rate in *Gdnf*$^{+/-}$ mice than in wild type mice. Altogether, these data support an important role for GDNF in the regulation of uptake, synthesis, and metabolism of DA during aging.
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1. Introduction

The decline in motor function associated with aging has been widely demonstrated in animal models of aging (Willig et al., 1987; Hebert and Gerhardt, 1998; Yurek et al., 1998; Zhang et al., 2000) and parallels a similar decline in human aging (Richards et al., 1993; Bennett et al., 1996; Kluger et al., 1997). Dopamine (DA) neuron dysfunction has been related to age-associated motor impairment in both humans (Volkow et al., 1998) and animals (Hebert and Gerhardt, 1998; Yurek et al., 1998; Gerhardt et al., 2002). The loss of functional DA neurons in the substantia nigra pars compacta (SNpc) and consequent loss of striatal DA are a hallmark of Parkinson's disease (PD) (Marsden, 1990), with its motor symptoms of bradykinesia, rigidity, and tremor (Hornykiewicz and Kish, 1987). The presence of some of these motoric deficits observed in many aged individuals has been termed age-related Parkinsonism (Bennett et al., 1996), and likely involves changes in the functional properties of DA neurons rather than neuronal loss as demonstrated in aged rats and monkeys (Yurek et al., 1998; Hebert and Gerhardt, 1999; Hebert et al., 1999; Grondin et al., 2003).

Glial cell line-derived neurotrophic factor (GDNF) is a member of the transforming growth factor-β superfamily

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(Lin et al., 1993). It has been hypothesized that age-related decreases in neurotrophic factor levels contribute to DA neuron degeneration and/or alterations in DA neuron function (Yurek and Fletcher-Turner, 2001). *In vivo* application of exogenous GDNF to the SN is reported to enhance DA neuron function in normal young (Hebert et al., 1996), aged (Hebert and Gerhardt, 1997; Grondin et al., 2003), and lesioned (Hoffer et al., 1994; Tomac et al., 1995) animals. In addition, GDNF is neuroprotective and neurorestorative in rat DA systems subjected to neurotoxic doses of methamphetamine (Cass et al., 2000; Cass et al., 2006) and produces functional restoration in rhesus monkeys exhibiting 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induced Parkinsonism (Gash et al., 1996; Grondin et al., 2002). GDNF has also been shown to enhance DA neuron function as indicated by 1) increased evoked DA release, 2) augmented locomotor behavior, 3) increased DA content in nigral tissue (Hebert et al., 1996; Hebert and Gerhardt, 1997), and 4) increased high affinity DA uptake (Lin et al., 1993). However, the exact role of GDNF in normal aging, specifically the maintenance of DA neuron system function, is not known.

To further elucidate the effects of GDNF, a knockout mouse model was created (Pichel et al., 1996) which exhibits a partial and stable reduction of the GDNF protein in brain tissues (Boger et al., 2006). At birth, midbrain DA systems in mice homozygous for the GDNF null mutation ($Gdnf^{-/-}$) appear unaffected (Moore et al., 1996); however, DA neuron function after the major apoptotic waves, which occur 2 and 14 days after birth (Mahalik et al., 1994), cannot be assessed because these mice die at birth due to kidney agenesis (Moore et al., 1996). Therefore, postnatal *in vivo* studies have used mice with a partial deletion of the *Gdnf* gene ($Gdnf^{+/-}$) (Boger et al., 2006). GDNF expression in the striatum appears to be critical for appropriate innervation, survival, and differentiation of midbrain DA neurons to their striatal targets during early development (Stromberg et al., 1993). Indeed, postnatal development of these DA neurons is compromised in the absence of GDNF (Granholm et al., 2000). Behavioral and immunohistochemical characterization of multiple age groups (4, 8, 12, 16, and 20 months of age) of $Gdnf^{+/-}$ mice compared with wildtype (WT) controls, provides evidence of a unique aging phenotype in the $Gdnf^{+/-}$ mice. A partial GDNF depletion leads to an earlier (12 months of age) loss of tyrosine hydroxylase positive (TH-positive) neurons in the SNpc as well as diminished spontaneous locomotor activity (Boger et al., 2006). The diminished locomotor activity and decline in TH-positive neurons also show an accelerated age-associated decline from 8 to 12 months in the $Gdnf^{+/-}$ mice while both measures in WT mice of the same age groups are unchanged (Boger et al., 2006). Young $Gdnf^{+/-}$ mice (6–9 months) exhibit no effect of GDNF reduction on spontaneous or methamphetamine-induced locomotor behavior (Gerlai et al., 2001; Boger et al., 2006; Boger et al., 2007). *In vitro* synaptosomal preparations from $Gdnf^{+/-}$ mice show increased dopamine transporter (DAT) activity, which has been suggested to predispose DA neurons to methamphetamine-induced toxicity (Boger et al., 2007). Consistent with the age related changes in DA systems noted above, DAT activity in the striatal pathway is altered in the normal aging process in animal models (Friedemann, 1992; Friedemann and Gerhardt, 1992; Hebert and Gerhardt, 1999; Hebert et al., 1999), and may be correlated with age-related motor deficits. Such dynamic changes in DA uptake using parameters associated with DAT activity have been characterized by *in vivo* chronoamperometry using carbon fiber microelectrodes (Gerhardt et al., 1986; Cass et al., 1993; Gerhardt et al., 1999). The high spatial (μm) and temporal (seconds) resolution of these techniques allows for the rapid and sensitive quantification of DA uptake in discrete DA terminal regions.

Based on previous *in vitro* findings that DA uptake in $Gdnf^{+/-}$ mice was greater than in WT mice (Boger et al., 2007), the present study investigated this age-associated deficit by using *in vivo* electrochemistry to address the effects of a partial and chronic genetic reduction of GDNF on the function of DA terminals in the striatum of middle-aged 12 month old $Gdnf^{+/-}$ mice. The following questions were addressed: (1) Do $Gdnf^{+/-}$ mice differ in behavioral response to DAT modulation via DAT inhibition? (2) Do $Gdnf^{+/-}$ mice exhibit altered DAT activity *in vivo*, and if so, what is the spatial pattern of this effect within striatal subregions? (3) Do $Gdnf^{+/-}$ mice have altered striatal dopamine D2 receptor expression? (4) Is whole tissue neurochemical content altered in $Gdnf^{+/-}$ mice? Thus, these studies focus on the functional properties of DA neurons in 12 month old $Gdnf^{+/-}$ mice that demonstrate accelerated age-related motor deficits and loss of TH-positive neurons as compared with WT littermate controls (Boger et al., 2006).

2. 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