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Neuronal nicotinic receptor agonists ameliorate spontaneous motor asymmetries and motor discoordination in a unilateral mouse model of Parkinson's disease

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ABSTRACT

The degeneration of the nigrostriatal dopamine (DA) system underlies the motor deficits in Parkinson's disease (PD). In recent years, epidemiological reports that smokers have lower incidences of PD have brought attention to the nicotinic acetylcholine system as a potential target for novel therapeutics. Nicotine, an agonist of neuronal nicotinic receptors (NNRs), modulates functions relevant to PD via stimulation of dopaminergic transmission in the nigrostriatal pathway, particularly via activation of α6β2* and α4β2* NNRs. Recently, reduced support of DA neurons by neurotrophic growth factors has been described in PD. Fibroblast growth factor (FGF) is critical for the development and protection of adult DA neurons. In FGF-2 knockout mice and the related *th-fgfr1(tk−)* mouse model there is heightened sensitivity to DA neuronal oxidative neurotoxin 6-hydroxydopamine (6-OHDA). In the present study, FGF-deficient transgenic mice *th-fgfr1(tk−)* were used to analyze the effects of novel full (TC-8831) and partial (TC-8581) agonists of β2-containing nicotinic receptors on impaired motor behavior following unilateral 6-OHDA lesions. The lesions generated spontaneous (drug-naïve) turning asymmetries that correlated exponentially with the depletion of DA biomarkers in the lesioned striata. These mice also exhibited a reduced capacity to remain on the accelerating rotarod. Oral administration of TC-8831, an NNR agonist with high specificity for β2 subunits and a full agonist at producing DA release from striatal synaptosomes, attenuated unidirectional turning and improved motor coordination. In contrast, partial β2 NNR agonist TC-8581 had no effect on behaviors in this model. This study demonstrates the potential of NNR targeting-compounds to facilitate motor function in PD.

1. Introduction

Parkinson's disease (PD) is characterized by the degeneration of dopamine (DA) neurons of the substantia nigra pars compacta (SNc) (Toulouse and Sullivan, 2008). These neurons innervate the striatum forming the nigrostriatal pathway, which is primarily responsible for the initiation and execution of movement (Rodriguez-Oroz et al., 2009). L-3,4-dihydroxyphenylalanine (L-DOPA) improves many PD motor symptoms, however, its side effects include unwanted dyskinesias, unpredictable fluctuations of motor response ("on/off" effects) and long-term impairments in synaptic plasticity and cognition (Grace, 2008; Jenner, 2008; Linazasoro, 2005).

An alternative therapeutic target for PD is the nicotinic acetylcholine receptor system. In recent clinical studies nicotine improved motor symptoms in advanced PD patients, particularly at high doses (Itti et al., 2009; Kelton et al., 2000; Lieberman et al., 2010; Villafane et al., 2007). Nicotine binds to neuronal nicotinic (acetylcholine) receptors (NNRs), ligand gated ion channels consisting of pentameric combinations of α (α2–α10) and β (β2–4) subunits (or all α7 subunits). NNRs localized on DA cell bodies and terminals mediate neuronal firing and DA release and regulate intracellular processes leading to changes in synaptic plasticity, gene transcription and neuroprotection (Dajas-Bailador and Wonnacott, 2004; Gotti and Clementi, 2004; Gotti et al., 2009; Grady et al., 1992; Salminen et al., 2004).

The most common types of NNR in the nigrostriatal system are the α4β2* and α6β2* subtypes (the * signifies the possible presence of other NNR subunits in the pentameric receptor complex), which are expressed in different stoichiometries. For example, α4β2* receptors

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can be present as $(\alpha4\beta2)_2\beta2$, with high sensitivity for agonists, as well as $(\alpha4\beta2)_2\alpha4$ and $(\alpha4\beta2)_2\alpha5$, combinations with lower sensitivity for agonists (Grady et al., 2010). In the nigrostriatal system, α6 associates with β2 in stoichiometries with and without the α4 subunit and the β3 subunit (reviewed in Letchworth and Whiteaker, 2011; Quik and Wonnacott, 2011). NNRs containing α4β2 subunits are localized on DA neurons throughout the brain (Champtiaux et al., 2003) and mediate approximately 60% of nicotine-mediated striatal DA release in rodents (Quik and McIntosh, 2006, Salminen et al., 2004). α6β2* NNRs have a more selective localization to striatal DA neurons (Champtiaux et al., 2003; Zoli et al., 2002). It was shown that α6β2* NNRs are critical for nicotine's effects on locomotion (Drenan et al., 2010; Gotti et al., 2010). Damage to the nigrostriatal system primarily affects α6β2* NNR expression, as lower declines in α4β2* and α7* NNRs have been reported (Quik and McIntosh, 2006; Yang et al., 2009). Nonetheless, α6β2* NNRs retain relatively normal function despite a high loss of DA innervation (Quik et al., 2001). Using fast cyclic voltammetry it was shown that both α4β2* and α6β2* NNRs mediate DA release after nigrostriatal lesions, however the α6β2* NNRs were more efficacious with increasing lesion severity (Perez et al., 2010). These findings suggest that targeting both types of NNRs (α4β2* and α6β2*), and particularly α6β2* subunits, after loss of DA terminals, has the potential to facilitate DA transmission and improve motor functions in PD.

Neurotrophic growth factors are critical for the development and protection of dopaminergic neurons (Lin et al., 1993; Nakao et al., 1996; Peterson and Nutt, 2008; Timmer et al., 2007, 2004). Impaired neurotrophic support of DA neurons by basic fibroblast growth factor (FGF) and related neurotrophins including brain-derived neurotrophic growth factor (BDNF), nerve growth factor (NGF) and glial-derived growth factor (GDNF) has been described in PD (Howells et al., 2000; Mogi et al., 1999; Nagatsu et al., 2000; Parain et al., 1999; Siegel and Chauhan, 2000; Tooyama et al., 1994). FGF distinguishes itself from other growth factors by its critical role in supporting the developing and adult DA system (Krejci et al., 2009; Mason, 2007). It was reported that FGF signaling increases DA levels in calbindin-negative SN neurons, those at greatest risk in PD patients (Murase and McKay, 2006). Furthermore, a reduction in the percentage of FGF-2 immunoreactive DA neurons was found in PD patients relative to healthy subjects (13% FGF-2 positive DA neurons versus 82% FGF-2 positive DA neurons in control brains) (Tooyama et al., 1994). In FGF-2 knockout mice, significantly fewer DA neurons survived compared to controls after 6-OHDA lesions, suggesting that reduced FGF signaling increases susceptibility to oxidative neurotoxins (Timmer et al., 2007). In the related *th-fgfr1(tk −)* model, mice generated with a developmental deficiency of FGF signaling have smaller and less-dense SNc DA neurons in adulthood (Klejbor et al., 2006), resembling the DA neuronal hypoplasia observed in PD patients (Ma et al., 1996; Moller, 1992). Unilateral 6-OHDA lesioning of these mice generated robust spontaneous asymmetrical turning and impaired motor coordination (Kucinski et al., 2012). The *th-fgfr1(tk −)* mouse and related models allow for the investigation of novel PD therapies on the background of reduced neurotrophic support of DA systems.

In the current study, the therapeutic potential of two novel nicotinic compounds (Fig. 1) possessing different affinities for α6β2* and/or α4β2* NNRs and DA release properties was assessed using *th-fgfr1(tk −)* mice with PD-related motor impairments. It was first determined that long-term (11 day) oral administration of TC-8831, a full agonist of α6β2/α4β2* NNRs at producing DA release from striatal synaptosomes, attenuated asymmetrical turning and increased latency before falling from the accelerating rod. In contrast, administration of TC-8581, a α6β2/α4β2* NNR compound that produces partial agonism of DA release, had no effect in this model. In a second experiment, constant delivery of TC-8831 via osmotic minipumps did not replicate the results observed with intermittent oral administration. These results suggest a potential therapeutic role of a novel NNR-targeting compound on motor impairments based on a transgenic/unilateral neurotoxin model of PD.

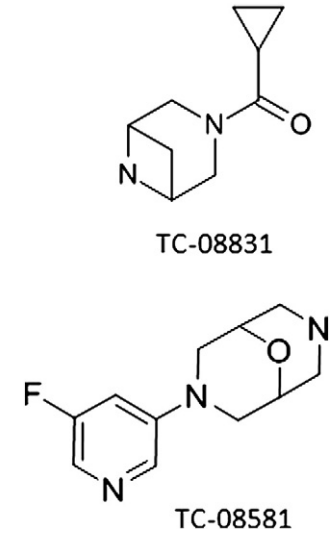

Fig. 1. Chemical structures of TC-8831 and TC-8581.

2. Materials and methods

2.1. Subjects

Homozygous transgenic *th-fgfr1(tk −)* mice express FGFR1(TK−) fused to rat TH gene promoter (4.5 kb) (Klejbor et al., 2006). The progenies were screened for the presence of the transgene by PCR amplification of tail DNA. Mice used in all specific experiments were selected from multiple litters. Mice (males and females) were singly housed throughout the course of the experiments and for at least four weeks prior to testing. The housing facility was maintained on a light: dark cycle of 12:12 h and animals had free access to food and water in their home cages. 87 mice (45 males and 42 females) were used in Experiment 1 and 48 mice (24 males and 24 females) were used in Experiment 2. All mice were between 4 and 8 months of age during the experiments.

All experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and with approval from the University at Buffalo Institutional Animal Care and Use Committee.

2.2. Drugs and chemicals

TC-8581 (DA release partial agonist) and TC-8831 (DA release full agonist) (see Tables 1 and 2) were obtained from Targacept, Inc. (Winston-Salem, North Carolina). The target dose selection for TC-8581 and TC-8831 in this assay was derived from the data collected with nicotine demonstrating efficacy in the model (significant effects at 2.4 mg/kg/d) (Kucinski et al., 2012) as well as preliminary tolerability assessments performed in wild-type mice (rotarod assessment) for

Table 1
Nicotinic receptor binding properties of TC-8581 and TC-8831.

Compound	hα4β2	hα6/3β2β3	hα7	hG	hM
TC-8581	1.8	22	76	790	53,000
TC-8831	3	20	620	270	470

TC-8581 and TC-8831 binding parameters using competitive in vitro binding assays. Data represent Ki values in nM of human receptors expressed in cells as described in the Materials and methods section.