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Negative results

Absence of regulator of G-protein signaling 4 does not protect against dopamine neuron dysfunction and injury in the mouse 6-hydroxydopamine lesion model of Parkinson's disease

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ABSTRACT

Regulator of G-protein signaling 4 (RGS4), a member of the RGS family of proteins that inactivate G-proteins, has gained interest as a potential drug target for neurological disorders, such as epilepsy and Parkinson's disease (PD). In the case of PD, the main current options for alleviating motor symptoms are dopamine replacement therapies, which have limitations because of side effects and reduced effectiveness over the long term. Research on new nondopaminergic PD drug targets has indicated that inhibition of RGS4 could be an effective adjuvant treatment option. The effectiveness of RGS4 inhibition for an array of PD-linked functional and structural neuroprotection end points has not yet been demonstrated. Here, we use the 6-hydroxydopamine (6-OHDA) lesioning model of the nigrostriatal pathway in mice to address this question. We observe, using a battery of behavioral and pathological measures, that mice deficient for RGS4 are not protected from 6-OHDA–induced injury and show enhanced susceptibility in some measures of motor function. Our results suggest that inhibition of RGS4 as a non-dopaminergic target for PD should be approached with caution.

1. Introduction

Parkinson's disease (PD) is a common movement disorder and, after Alzheimer's disease, the most frequent progressive neurodegenerative disorder (Shastry, 2001). It is characterized by a loss of dopaminergic neurons and debilitating motor and nonmotor symptoms (Sprenger and Poewe, 2013). No disease-modifying or preventive therapies exist, and current therapeutics only alleviate symptoms by counteracting dopamine loss (Poewe, 2009). However, the benefits of these treatments are limited by a gradual loss of efficacy and long-term adverse effects (Jankovic and Aguilar, 2008). One of the recently proposed nondopaminergic drug targets is RGS4, a GTPase accelerating protein for specific G-protein coupled receptors (De Vries et al., 2000). Increased RGS4 activity following disease-related dopamine loss has been suggested to lead to PD-associated dysfunction of neuronal projections to the globus pallidus and substantia nigra (SN) (Di Marzo et al., 2000; Lerner and Kreitzer, 2012). A first indication that RGS4 may be a target for PD was based on the observation that mice lacking the *Rgs4* gene ($Rgs4^{-/-}$ mice) were functionally less impaired than wild-type controls after 6-OHDA lesioning of their nigrostriatal pathway (Lerner and Kreitzer, 2012). To investigate if RGS4 inhibition provides protection against injury-induced loss of nigral dopaminergic neuron structure and function, focusing on the evaluation of RGS4 as a target for PD, an aspect that has not been investigated in-depth previously, we measured behavioral and neuropathological effects of intracerebral 6-OHDA administration in $Rgs4^{-/-}$ mice and their littermate wild-type controls (Grillet et al., 2005). Since mice lacking both alleles of a gene can compensate for this lack and do not always have the same phenotype or response to injury as heterozygotes (Klamer et al., 2005), we also analyzed heterozygous RGS4 mice ($Rgs4^{+/-}$). We found that motor function was

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significantly impaired by 6-OHDA in both *Rgs4*$^{-/-}$ and *Rgs4*$^{+/-}$ mice, with *Rgs4*$^{-/-}$ mice even showing motor deficiencies in tests that showed no or only slight impairment of *Rgs4*$^{+/+}$ wild-type controls. We also observed that neither tyrosine-hydroxylase (TH) positive fibers in the striatum nor TH-positive neurons in the SN were protected against 6-OHDA–induced injury. Our results contrast with those of a previous study (Lerner and Kreitzer, 2012) and indicate that *Rgs4* gene deletion does not always protect against toxin-induced nigral neuron dysfunction and injury in mice.

2. Methods

All experiments were approved by the institutional animal experimentation ethics committee and by appropriate government agencies. Heterozygote *Rgs4* (*Rgs4*$^{+/-}$) mice were purchased from Jackson (#005833; https://www.jax.org/strain/005833) and bred with C57BL/6J wild-type mice from the same vendor to obtain study cohorts (see Supplementary Material, Materials and Methods section, for details on genotyping). Mice were used at 12–18 weeks of age (equal numbers of males and females) and euthanized after the in-life procedures. Standard protocols were used according to the reference publications for stereotactical unilateral intrastriatal 6-OHDA injections (Bagga et al., 2015), motor behavior measurements [cylinder test (Glajch et al., 2012), pole test (Matsuura et al., 1997), and grip strength (Ferguson et al., 2015)], TH immunostaining, and quantitative image analysis of striatal and nigral TH-positive neurons (Masliah et al., 2000), except for minor modifications as detailed in the Supplemental Material. The morphometric quantitation of TH-positive nigral neurons was validated by stereological assessment on a separate set of tissues (see Supplementary Material and Supplementary Fig. 2). For statistical evaluation, all variables were first tested for normality (Shapiro-Wilk, D'Agostino-Pearson, and Kolmogorov-Smirnov). Since for each type of measurement, the normality assumption was rejected at least once, the nonparametric Kruskal-Wallis test was used for multiple comparisons, followed by the Mann-Whitney test for pairwise group comparisons. Control groups were either wild-type mice before surgery or the wild-type mice after injection with vehicle. The *p*-values below 5% were considered significant.

3. Results

We observed no gross neurological, developmental, or organ deficiencies in heterozygous (*Rgs4*$^{+/-}$) or homozygous (*Rgs4*$^{-/-}$) mice compared to their wild-type littermate controls (*Rgs4*$^{+/+}$). The segregation of genotypes in the study cohorts was Mendelian. Weights of mice in all 3 genotypes were similar (Supplementary Fig. 1A and B). In 3 different behavioral motor assessments (pole, grip strength, and cylinder tests), *Rgs4*$^{-/-}$ and *Rgs4*$^{+/-}$ mice showed no significantly different performance in comparison to *Rgs4*$^{+/+}$ controls (Supplementary. Fig. 1A–F). The integrity of their nigrostriatal pathway, measured by TH immunostaining, was also similar to that of their *Rgs4*$^{+/+}$ littermates (Supplementary Fig. 1G). This allowed us to use these mice to study the effects of RGS4 deficiency on experimental PD-related disease outcomes. After determining

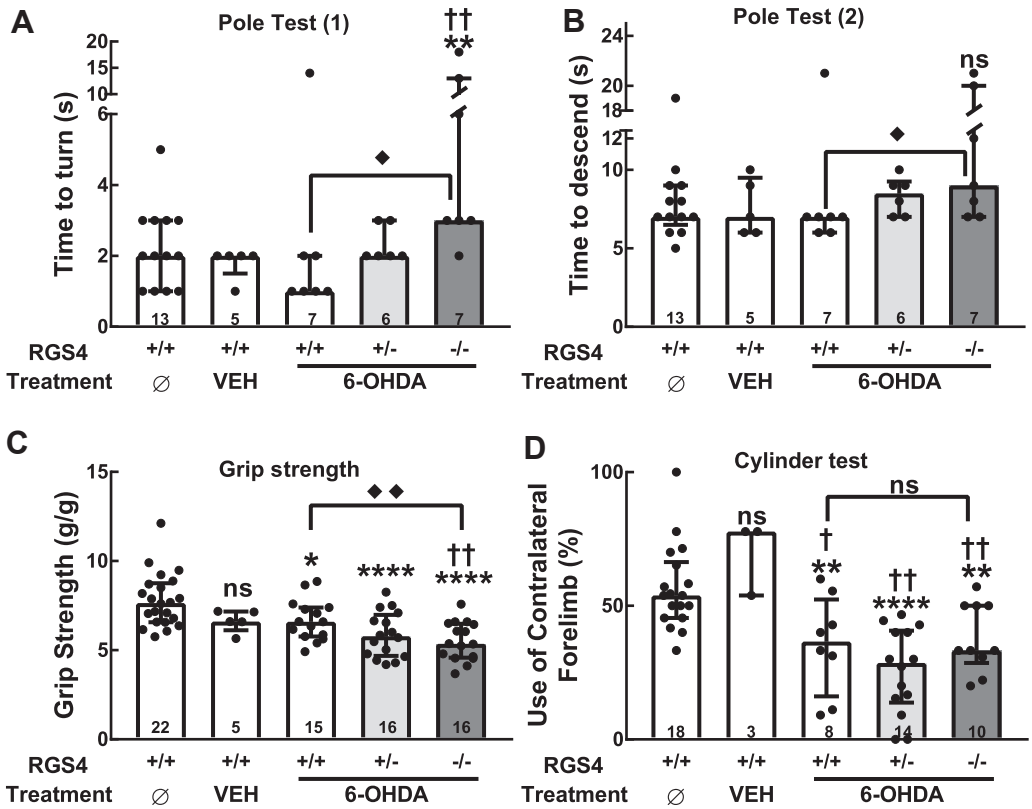

Fig. 1. RGS4 deficiency in mice does not protect against motor impairment induced by intrastriatally administered 6-OHDA. (A) Pole test (1): time to turn on pole top; (B) pole test (2): time to descend from pole; (C) grip strength test; and (D) cylinder test. The bars represent the group medians +/− the interquartile ranges (data were not normally distributed). Individual data points are shown as dots. The numbers at the bottom of the bars represent the numbers of mice in each group. Ø represents all the wild-type mice (*Rgs4*$^{+/+}$) at baseline (before or without lesioning), VEH indicates vehicle injected *Rgs4*$^{+/+}$ mice. Then, 2 μg of 6-OHDA was administered to mice of all 3 genotypes. Two mice in the VEH wild-type group did not perform the cylinder test and had to be excluded, hence the small group size. See main text and Supplementary Material for details. *, **, and **** represent $p <$ 0.05, <0.01, and <0.0001 compared to baseline *Rgs4*$^{+/+}$ (Ø); † and †† represent $p < 0.05$ and <0.01 compared to VEH-treated *Rgs4*$^{+/+}$; ◆ and ◆◆, represent $p < 0.02$ and <0.01 6-OHDA–treated *Rgs4*$^{+/+}$ versus *Rgs4*$^{-/-}$, ns = not significant; all comparisons by Mann-Whitney test.