GlyT1 Inhibitors Promote Dopaminergic Striatal Sprouting

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INTRODUCTION

NMDA receptor activity is involved in shaping synaptic connections throughout development and adulthood. We found that brief activation of NMDA receptors in cultured ventral midbrain dopamine neurons enhanced their axon growth rate and induced axonal branching (Schmitz et al. 2009 J. Neurosci. 29: 11973). Here, we examined in a toxin-based Parkinson’s Disease mouse model, if activation of NMDA receptors would promote growth of spared dopaminergic axons into the dopamine-denervated striatum. We used a pharmacological approach to enhance NMDA receptor-dependent signaling (A) by treatment with an inhibitor of glycine transporter-1 (B), ACPPB (C), that elevates levels of extracellular glycine, a co-agonist at NMDA receptors.

RESULTS

1. Unilateral 6-OHDA model: lesioned dorsal, intact ventral striatum

To study dopaminergic reinnervation of the striatum, we chose a 6-OHDA dose (9-11 μg) for intrastriatal injection that caused more than 70% cell death in the substantia nigra, but less than 50% in the ventral tegmental area (VTA, A: sections stained for TH) such that at 3 weeks post-lesion the dorsal striatum was mostly devoid of dopamine fibers, whereas the ventral striatum was spared (B, stained for DAT). Mice were treated with the GlyT1 inhibitor ACPPB from week 3 to week 7 post-lesion (C).

2. GlyT1 inhibitor (ACPPB) treatment enhanced dopaminergic reinnervation of dorsal striatum

Reinnervation of the dorsal striatum was examined at 7 weeks post-lesion in cryosections stained for the dopamine transporter (DAT, A, arrows indicate dorsal-most fibers). Structures resembling axonal growth cones were found in the dorsal striatum (B). Axon density was quantified as area coverage normalized to that of the unlesioned hemisphere in 4 dorsoventrally arranged regions as indicated (1-4). Axon density in the more medial and ventral striatal regions (3 and 4) was about two-fold higher in ACPPB-treated mice (C).

3. ACPPB treatment abolished lateralization of sensory-motor behavior

In the “corridor test” unilaterally lesioned mice retrieved sugar pellets from containers preferentially on the side of the lesioned hemisphere (A). Mice were tested at 3 and 7 weeks post-lesion. There was significant improvement in lateralization from 3 to 7 weeks in both untreated and treated mice (B and C), but in treated mice recovery was significantly more pronounced with complete recovery from lateralization in most cases.

4. Dopamine tissue content was increased in ACPPB-treated mice

Tissue content of norepinephrine, DOPAC, dopamine and serotonin was measured in striatal tissue from untreated and treated mice 7 weeks post-lesion. DA and DOPAC content was significantly higher in ACPPB-treated mice (A). Behavior (B) was only slightly correlated with DA content (C).

5. Effects of ACPPB depended on NMDA receptors in dopamine neurons as revealed by conditional NR1 knock out mice

To test for the successful knockdown of NMDA receptors in dopamine neurons, we recorded firing rates in response to NMDA in brain slice preparations (A). Neurons from conditional NR1 KO mice (Slc6a3−/−Cre/+/Nigra-loxp/lacz) did not display an increase in firing rate, in contrast to cells in slices derived from DAT Cre (Slc6a3−/−Cre/+ (B)). Treatment with ACPPB caused increased striatal reinnervation in DAT Cre mice (C and green columns in D) compared to C57BL/6J mice (blue columns in D). In conditional NR1 KO (cNR1 KO) mice, however, ACPPB treatment did not increase axon density (C and red columns in D), although there was a basal level of reinnervation at 7 weeks. This indicates that ACPPB effects on the recovery from 6-OHDA lesions depended on the presence of NMDA receptors in dopamine neurons.

CONCLUSIONS

Dopamine neuron axon growth is regulated by axonal NMDA receptors.

Following 6-OHDA lesion, the dorsal striatum is reinnervated by spared dopamine axons from the ventral striatum. This reinnervation is enhanced by GlyT1 inhibitors that augment NMDA receptor transmission by elevating levels of the co-agonist glycine.

The GlyT1 inhibitor-promoted reinnervation leads to recovery of normal motor function.

GlyT1 inhibitors could provide a means to treat Parkinson’s Disease, if such functional sprouting could be induced in areas that are spared in PD (i.e., the caudate nucleus, medial portions of the putamen, and the nucleus accumbens).

GlyT1 inhibitors are being developed for treatment of schizophrenia. (The Roche compound RG1678 is in phase III clinical trials.)

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