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Research Report

Cortical glutamate levels decrease in a non-human primate model of dopamine deficiency

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ABSTRACT

While Parkinson's disease is the result of dopaminergic dysfunction of the nigrostriatal system, the clinical manifestations of Parkinson's disease are brought about by alterations in multiple neural components, including cortical areas. We examined how 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) administration affected extracellular cortical glutamate levels by comparing glutamate levels in normal and MPTP-lesioned nonhuman primates (*Macaca mulatta*). Extracellular glutamate levels were measured using glutamate microelectrode biosensors. Unilateral MPTP-administration rendered the animals with hemiparkinsonian symptoms, including dopaminergic deficiencies in the substantia nigra and the premotor and motor cortices, and with statistically significant decreases in basal glutamate levels in the primary motor cortex on the side ipsilateral to the MPTP-lesion. These results suggest that the functional changes of the glutamatergic system, especially in the motor cortex, in models of Parkinson's disease could provide important insights into the mechanisms of this disease.

1. Introduction

In idiopathic Parkinson's disease (PD), motor deficits are believed to be a result of dopamine deficiency in the motor circuit, comprised of the supplementary motor area, parts of the premotor cortex and the primary motor cortex (Alexander et al., 1990; Parent and Hazrati, 1995; Wichmann and DeLong, 1993, 2003, 2008). The cortical changes in these regions are

Abbreviations: PD, Parkinson's disease; MEA, microelectrode array; GluOx, glutamate oxidase; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; TH, tyrosine hydroxylase; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

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considered to be some of the hallmark symptoms of neurodegenerative diseases including PD (Kuninobu et al., 1993; Lefaucheur, 2005; Sabatini et al., 2000). In PD, dopaminergic neurons in the substantia nigra undergo a loss of function that indirectly results in diminished activity through the basal ganglia-thalamo-cortical pathway that leads to a subsequent decrease in glutamatergic output from the motor cortex (Wichmann and DeLong, 1996).

Motor symptoms of PD are largely attributed to the imbalance of inhibitory and excitatory processes in the motor cortex and subcortical neuronal circuits after a nigrostriatal dopamine deficit (Ridding et al., 1995). Because glutamate is the principal excitatory neurotransmitter in the CNS, understanding the function of glutamate neurotransmission may provide important insight into understanding PD. Glutamate in the brain acts through ionotropic (NMDA, kainite and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, AMPA) receptors and G-protein-coupled metabotropic receptor subtypes (Bai et al., 2004; Hof et al., 2002). In the cerebral cortex, about 80% of neurons are glutamatergic pyramidal neurons and the remaining 20% of neurons are GABAergic nonpyramidal interneurons with axons confined to the cortex. Thus, all outputs of the cerebral cortex arise from glutamatergic pyramidal neurons located in a specific arrangement of layers and project to defined targets. Corticostriatal glutamatergic afferents and mesostriatal dopaminergic afferents commonly converge onto the same postsynaptic spines of medium projection neurons in which the vast majority of striatal neurons are GABAergic. Thus, this synaptic triad may be a crucial element in the striatum's regulation of glutamatergic cortical information flow (Bamford et al., 2004).

In PD, dopamine depletion leads to a complex cascade of events that produce an excessive inhibition of the thalamocortical glutamatergic pathway. Glutamatergic mechanisms are critically involved in determining both dendritic spine development and maintenance (Bloodgood and Sabatini, 2008; Korkotian and Segal, 2001; Lippman and Dunaevsky, 2005; McKinney, 2005; Passafaro et al., 2003). Presently, only a few studies have directly examined resting glutamate levels in a living nonhuman primate's brain, especially in one showing parkinsonian symptoms. To determine if cortical basal glutamate levels could be altered by dopaminergic dysfunction (MPTP-induced lesions), we employed electrochemical detection by using amperometry in conjunction with enzyme-based microelectrodes to characterize extracellular levels of glutamate in hemiparkinsonian monkeys.

2. Results

2.1. *Verifying dopaminergic depletion*

In addition to behavioral assessment with the nonhuman primate Parkinsonian rating scale, we used post-mortem analyses to verify the effectiveness of the MPTP lesion in depleting dopaminergic neurons from the nigral-striatal pathway. After staining sections of the substantia nigra for tyrosine hydroxylase (TH), the enzyme that converts the dopamine precursor L-DOPA to dopamine, we identified 85% fewer TH positive (TH^+) dopamine neurons in the MPTP-lesioned substantia nigra (27,950±3722 TH^+ cells) versus the un-lesioned side (186,877±2472 TH^+ cells; $t(5)=31.6$, $p<0.0001$). Meanwhile, to determine the effect of dopamine denervation at the cortical level, we assessed the TH^+ fiber density and found significantly lower levels in the MPTP-lesioned side than the unlesioned side (Fig. 1; $F_{(1,10)}=17.68$; $p=0.0018$). Sidak multiple-comparison tests revealed significant less TH^+ fiber density in the MPTP-lesioned side premotor cortex (1.4±0.11% of total fields) than the contralateral side (1.9±0.14; $p<0.05$). We found similar reductions in the motor cortex (1.3±0.29 MPTP-lesioned side vs. 1.8±0.32 contralateral side; $p<0.05$). These results confirm that the MPTP lesion had produced a characteristic dopamine depletion of the nigrostriatal pathway.

2.2. *Basal glutamate levels in the primary motor cortex*

We measured basal glutamate levels in the motor cortex using glutamate biosensors (Fig. 2). Basal glutamate levels in the motor cortex on the side ipsilateral to MPTP administration were significantly lower than on the contralateral side (Fig. 3A). The average basal glutamate levels were 23% lower in the motor cortex on the lesioned side (6.3±2.7 μM) than in the same regions on the unlesioned side (8.1±2.8 μM; $t(5)=2.97$, $p=0.031$, [NHP #1: 1.0 μM vs. 1.5 μM, NHP #2: 5.0 μM vs. 1.9 μM, NHP #3: 8.9 μM vs. 5.1 μM, NHP #4: 20.6 μM vs. 18.6 μM, NHP #5: 9.8 μM vs. 8.4 μM, NHP #6: 3.2 μM vs. 2.0 μM, unlesioned vs. MPTP-lesioned side, respectively]). Next, when we examined glutamate levels from individual recording coordinates in the motor cortex, we observed that, again, glutamate levels were lower on the MPTP-lesioned side than in the unlesioned side, but not different from each other from medial to lateral (Fig. 3B, two-way repeated measures ANOVA, main effect of the lesion $F(1,4)=23.33$, $p=0.0085$, no main effect of the coordinate location $F(4,16)=0.86$, $p=0.50$ or interaction $F(4,16)=0.3545$, $p=0.84$). When we compared basal glutamate levels in MPTP-lesioned animals to basal glutamate levels in control animals, we found that administration of MPTP resulted in a non-significant reduction of basal glutamate levels (6.3±2.7 μM, $N=6$) in the cortex compared with normal animals (12.5±5.0 μM; $N=8$, $p=0.34$, Fig. 4A). Finally, slightly higher basal glutamate levels were observed in the same-side hemisphere of normal age-matched

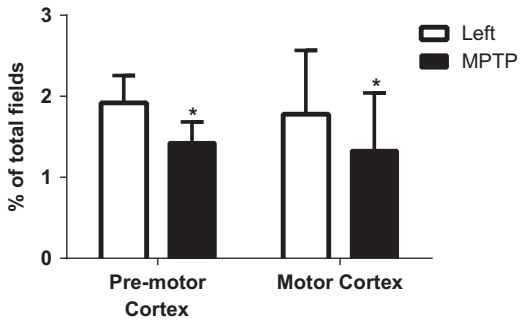

Fig. 1 – TH^+ fiber densities in the pre-motor and motor cortices. TH^+ fiber densities were approximately 25% lower in the MPTP-lesioned-side than the contralateral side in the pre-motor and motor cortex. *=$p<0.05$.