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INTRASTRIATAL INJECTION OF IONOMYCIN PROFOUNDLY CHANGES MOTOR RESPONSE TO L-DOPA AND ITS UNDERLYING MOLECULAR MECHANISMS

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Abstract—Long-term L-DOPA treatment of Parkinson's disease is accompanied with fluctuations of motor responses and L-DOPA-induced dyskinesia (LID). Phosphorylation of the dopamine and c-AMP regulated phosphoprotein of 32 kDa (DARPP-32) plays a role in the pathogenesis of LID, and thus dephosphorylation of this protein by activated calcineurin may help reduce LID. One important activator of calcineurin is the Ca^{2+} ionophore ionomycin. Here, we investigated whether intrastriatal injection of ionomycin to hemiparkinsonian rats produced changes in L-DOPA responses including LID. We also analyzed the effects of ionomycin on key molecular mediators of LID. Results confirmed our hypothesis that ionomycin can down-regulate the phosphorylation of DARPP32 at Thr-34 and reduce LID. Besides, ionomycin decreased two established molecular markers of LID, FosB/ΔFosB and phosphorylated ERK1/2. Ionomycin also decreased the phosphorylation of three main subunits of the NMDA receptor, NR1 phosphorylated at ser896, NR2A phosphorylated at Tyr-1325, and NR2B phosphorylated at Tyr-1472. Furthermore, the anti-LID effect of striatally injected ionomycin was not accompanied by reduction of the antiparkinsonian action of L-DOPA. These data indicate that ionomycin largely interacts with striatal mechanisms that are critical to the L-DOPA motor response highlighting the role of protein dephosphorylation by calcineurin.

Key words: DARPP-32, dyskinesia, ionomycin, L-DOPA, NMDA.

INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disease which is typically manifested by motor symptoms that are principally caused by the loss of dopaminergic neurons in the substantia nigra (SN) (Jenner et al., 2013). Dopamine replacement with the dopamine precursor L-DOPA is still the most effective treatment for PD. However, with time L-DOPA treatment is complicated by motor response fluctuations and involuntary choreic and dystonic movements called LID. These complications can appear after a few years of treatment and affect nearly 90% of the patients after 9-years of treatment (Bhidayasiri and Truong, 2008). Although the underlying mechanisms of LID remain elusive, several molecules play definitely a role. One of these molecular markers is ΔFosB, a transcription factor that is markedly increased in the striatum following chronic L-DOPA treatment in animal models and in patients (Doucet et al., 1996; Vallone et al., 1997; Andersson et al., 1999; Ruiz-DeDiego et al., 2015). Furthermore, transgenic overexpression of ΔFosB in the striatum reproduces dyskinesia in animals that have not been chronically exposed to L-DOPA (Cao et al., 2010). This transcription factor regulates the expression of multiple genes, which may be involved in the molecular pathways of LID; however, these genes remain to be identified. Another well-recognized molecular marker of LID is phosphorylated extracellular signal-regulated kinases (p-ERK). The p-ERK levels are increased in the striatum following chronic L-DOPA administration in rats and monkeys, and p-ERK down-regulation can attenuate LID (Pavon et al., 2006; Darmopil et al., 2009). A critical mechanism mediated by p-ERK is the phosphorylation of dopamine- and cAMP-regulated phosphoprotein of 32 kDa (DARPP-32) (Valjent et al., 2005). DARPP-32 phosphorylated at Thr-34 is known to participate in the molecular signaling cascade associated with LID pathogenesis.

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Abbreviations: AIMs, abnormal involuntary movement scale; ANOVA, analysis of variance; CPU, striatum; CREB, cAMP response element-binding protein; DARPP32, dopamine- and c-AMP-regulated phosphoprotein of 32 kDa; DREAM, calcium-binding protein downstream regulatory element antagonistic modulator; ECL, Electro-Chemi-Luminescence; IHC, immunohistochemistry; LID, L-DOPA-induced dyskinesia; LTD, long-term depression; LTP, long-term potentiation; MFB, medial forebrain bundle; PD, Parkinson's disease; p-ERK, phosphorylated extracellular signal-regulated kinases; PFA, paraformaldehyde; PKA, protein kinase A; pp-1, protein phosphatase 1; SDS-PAGE, SDS–polyacrylamide gel; SN, substantia nigra; TH, tyrosine hydroxylase; WB, Western blotting.

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Dopamine binding to the D1 receptor signals the activation of protein kinase A (PKA), although PKA can also be activated by other neurotransmitters such as glutamate. Activated PKA phosphorylates DARPP-32 at Thr34, which is a potent inhibitor of protein phosphatase 1 (pp-1) (Fienberg et al., 1998); therefore, p-DARPP-32 regulates the phosphorylation of downstream signaling molecules (Snyder et al., 1998; Greengard et al., 1999). In addition, there is evidence that p-DARPP-32 participates in the phosphorylation of NMDA receptors, especially the NR1 subunit through inhibition of pp-1 (Snyder et al., 1998). NMDA receptor antagonists have significant motor effects in animal models of PD (Boldry et al., 1995; Loschmann et al., 2004; Papa et al., 2004) including anti-LID effects (Papa and Chase, 1996; Hadj Tahar et al., 2004; Wessell et al., 2004). In addition, the NMDA antagonist amantadine is currently in clinical use to treat LID. Notably, the phosphorylation of NR2B-Y1472 can be induced by activation of dopamine D1 receptor in mature hippocampus and cortex (David et al., 2014), suggesting a PKA/DARPP-32 mechanism. Chronic treatment with L-DOPA leads to prominent hyperphosphorylation of NR1 at serine residues, and NR2A and NR2B at tyrosine residues (Dunah et al., 2000). Furthermore, L-DOPA treatment changes the expression, subcellular distribution and phosphorylation of NMDA receptors (Calon et al., 2003; Hallett et al., 2005). Altogether these data suggest an important role of the NMDA receptor signaling and its regulation by phosphorylating mechanisms in the pathogenesis of dyskinesia.

Clearly, p-DARPP-32 that inhibits protein phosphatase-1 is an important contributor to the increase in phosphorylated subunits of NMDA receptors. The striatal level of p-DARPP-32 correlates with the severity of LID in rodents (Santini et al., 2010), and DARPP-32-deficient mice exhibit low dyskinesia expression (Santini et al., 2007). Conceivably, inactivation of DARPP-32 may reduce the expression of LID. Activated calcineurin is a serine/threonine phosphatase that can dephosphorylate several proteins including calcium channels, NMDA receptors, AMPA receptors, the cAMP response element-binding protein (CREB), synapsin I and DARPP-32 (Nishi et al., 1997; Greengard et al., 1999; Jovanovic et al., 2001; Winder and Sweatt, 2001). Additionally, calcineurin is involved in modulating synaptic plasticity mechanisms, long-term depression (LTD) and long-term potentiation (LTP) that are altered in LID (Picconi et al., 2003). Because calcineurin is activated by the Ca^{2+} ionophore ionomycin, we hypothesize that ionomycin (Du et al., 2008) via calcineurin activation and desphosphorylation of p-Thr34-DARPP-32 may have effects on LID. We used the rodent model of abnormal involuntary movement scale (AIMs) induced by chronic L-DOPA administration to rats with unilateral 6-OHDA lesion of the nigrostriatal system to investigate ionomycin's effects on LID and its underpinning molecules.

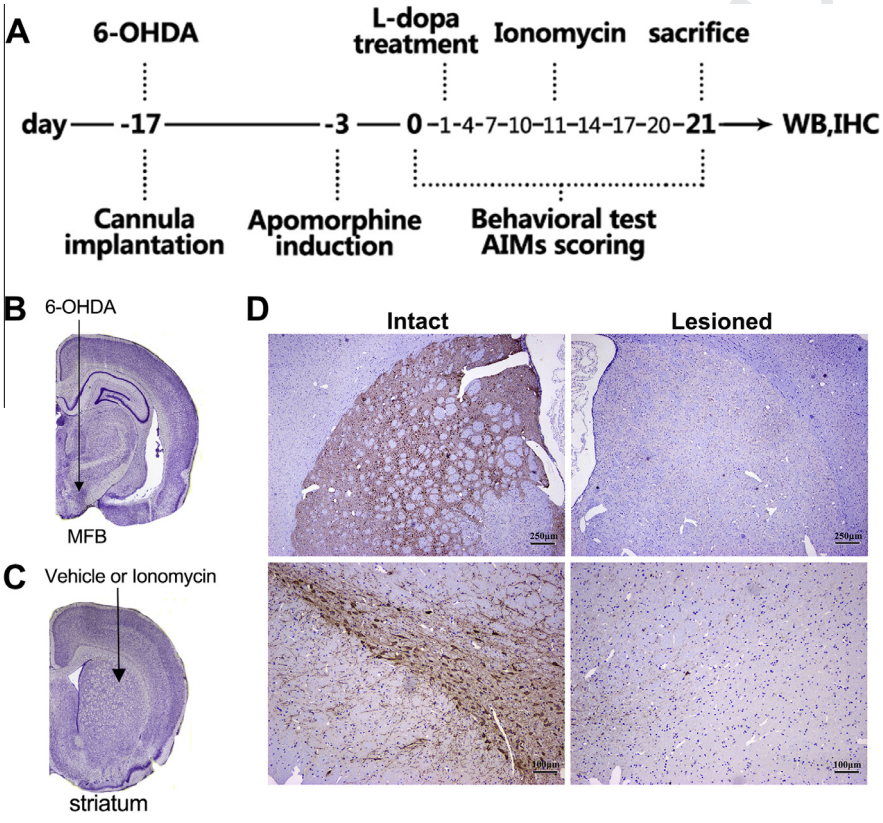

Fig. 1. Schematic illustration of experimental designs and tyrosine hydroxylase immunostaining of substantia nigra (SN) and striatum in the rat 6-OHDA lesion model. (A) Schematic representation of the timeline of experiments. (B and C) Rat subjected to 6-OHDA lesion in the medial forebrain bundle (MFB) and a cannula implanted in the striatum followed by injection of vehicle or ionomycin. (D)The nigrostriatal lesion was additionally verified by TH immunochemistry in the SN (100×) and striatum (40×). Scale bars are indicated in the graph.

EXPERIMENTAL PROCEDURES

Animals

The experimental design and operational is depicted in Fig. 1A. Fifty male Sprague–Dawley rats weighing 200–250 g (Experimental Animal Center, Tongji Medical College, Huazhong University of Science and Technology) were maintained in SPF animal house under 12 h light/dark with water and food available ad libitum. All the experimental protocols conform to the Ethics Committee of Huazhong University of Science and Technology. After acclimation for a few days, six rats were selected for the normal group, the other remaining 44 animals were anesthetized with 7% chloral hydrate (0.5 ml/kg) and injected with 5 μl 6-OHDA (Sigma–Aldrich, 2 μg/μl, dissolved in 0.2% ascorbic acid, Fig. 1B) in the medial forebrain bundle (MFB) as described elsewhere (Lindgren

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