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Modulation of CaMKIIa-GluN2B interaction in levodopa-induced dyskinesia in 6-OHDA-lesioned Parkinson's rats

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ABSTRACT

Long-term treatment with L-dopa leads to involuntary aimless movements called L-dopa-induced dyskinesia (LID) has hindered its use in Parkinson's disease (PD) patients. Emerging evidence suggests a possible role of CaMKIIa and its interacting partners in the development of LID. In this study, we found that CaMKIIa was found to form complexes with GluN2B after chronic administration of L-dopa in adult rat striatal neurons. Intrastriatal injection of KN-93 significantly reduced the level of GluN2B in CaMKIIa precipitates with a dose dependent response, as well as reduced the Global ALO AIM score without ablation of the therapeutic response to L-dopa. In parallel, intrastriatal injection of MK-801 significantly alleviated the level of CaMKIIa in GluN2B precipitates compared to LID group ($p < 0.01$), and this is accompanied by realizing improvement of the Global ALO AIM score also without affect the efficacy of L-dopa. In summary, the present study indicated that CaMKIIa-GluN2B interaction had an important role in the development of LID. Disrupt of this link by intrastriatal infusion of KN-93 or MK-801 ameliorated dyskinesia in 6-OHDA-lesioned PD rats.

1. Introduction

Parkinson's disease (PD) is a complicated neurodegenerative disease and is associated with a mass of pathological features and various symptoms that affect organs throughout the body [1]. It is a pathology that develops over many years and where complex functional modifications within the basal ganglia circuitry are triggered by the progressive loss of pigmented nigrostriatal dopaminergic neurons and the presence of Lewy bodies [2]. Until now, there is no disease-modifying therapy that has been testified to be effective to prevent its progression. Treatment of PD is still based on the use of L-3,4-dihydroxyphenylalanine (L-dopa), which has been regarded as the gold-standard treatment for over many years [3]. Compared with other available dopaminergic treatments, L-dopa therapy is featured with the greatest improvement in motor function [4]. However, chronic use of L-dopa leads to aimless involuntary movements, which is called L-dopa-induced dyskinesia (LID) and is argued to result from the molecular and ultrastructural changes in medium spiny neurons due to pulsatile nature of L-dopa kinetics induced [5]. LID are hard to control and manage once they have developed in patients.

Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), an enzyme that is ubiquitously expressed in the central nervous system, acts to increases intracellular Ca^{2+} concentrations by fascinating Ca^{2+}-induced augmentation of L-type Ca^{2+} current [6]. This kinase has long been known to be essential for synaptic plasticity and is involved in learning and memory process. Recent research points to a new field of its function in the nervous system by revealing that CaMKII also plays an important role in the synaptic organization [7]. In addition, CaMKII is also known to be associated with the transformation, neurotransmitter synthesis and release, and postsynaptic receptor signaling [8]. Moreover, one previous study demonstrated that CaMKII also participates in serine phosphorylation of both NMDA and AMPA receptors. Inhibition of CaMKII suppresses motor-response changes in 6-OHDA-lesioned rats [9].

CaMKII is composed of four different chains: alpha, beta, gamma, and delta. Among them, CaMKIIa is one of the major forms of CamKII. It has been found to play a critical role in sustaining activation of CamKII at the postsynaptic density [10]. Emerging evidence suggests a possible role of CaMKIIa in LID or PD. Zhang et al have showed that pharmacological inhibition of CaMKIIa with a selective inhibitor KN-93 was beneficial in reducing the occurrence of LID in PD rats, probably because it can lower the expression of pGluR1 via suppressing the activation of CaMKIIa [11]. Moreover, it was also confirmed in a previous study of our own group that L-dopa induced an increase in CaMKIIa-D2R [12], which is reversed by intrastriatal injection of CaMKIIa-D2R interaction-dead peptide. The result revealed an interaction between

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CaMKIIa and D2R in striatal neurons which is sensitive to long-term administration of L-dopa in LID models. Meanwhile, Liu et al reported that dopamine stimulation by cocaine enhances the formation of a heteroreceptor complex between D2R and NMDA receptor GluN2B (N-methyl D-aspartate receptor subtype 2B) subunits in the striatal neurons in vivo [13]. Focus on interactions between the NMDA glutamate receptor and dopamine D1 and D2 receptors, and address the potential value of receptor heteromers in seeking novel therapeutic targets [14]. However, in animal models of LID, it is still unknown whether CaMKIIa interacts with GluN2B, and if so, whether CaMKIIa-GluN2B interactions are sensitive to L-dopa and contribute to the development of LID.

In the present study, therefore, we examined the relationship between CaMKIIa and GluN2B in vivo. We first investigated the levels of CaMKIIa, phosphor-CaMKIIa and GluN2B proteins in each group After confirmation of the combination between CaMKIIa and GluN2B in vitro directly, we carried out behavioral experiments to determine the role of CaMKIIa- GluN2B interactions in LID models. We also used the CaMKIIa inhibitor (KN-93: 5 ug/kg and 10 ug/kg) and GluN2B inhibitor (MK-801: 0.1 mg/kg and 0.3 mg/kg) to disrupt the binding between CaMKIIa and GluN2B proteins to explore the involvement of CaMKIIa-GluN2B interactions in the development of LID.

2. Materials and methods

2.1. Animals

This study was conducted on Eight-eight male Sprague-Dawley (SD) rats (Shanghai and Wenzhou; the people republic of China; weight 200–250 g). Rats were used to study the pharmacological blockade of CaMKIIa or GluN2B in order to further investigate the interaction between CaMKIIa-GluN2B in the dyskinetic response. All animals were provided by Wenzhou Medical University animal facility and divided into several cages with a maximum of four rats per cage as described previously [15]. All experimental protocols involving the animals were reviewed and approved by the Ethical Committee of the Medical School of Wenzhou medical University and Shanghai Jiaotong University. All procedures were carried out in accordance with the approved guidelines and regulations of the National Institutes of Health for the care and use of laboratory animals. In this study, no pre-registered and no blinding was performed and no sample size calculation and power analysis was performed.

2.2. Induction of L-dopa-induced dyskinesia (LID)

Unilateral 6-OHDA (hydroxydopamine) lesioned PD models were performed according to our previous study based on the standard protocol [16]. Briefly, rats were anesthetized with 10% chloral hydrate (0.35 ml/100 g) and installed on a digitalized stereotaxic apparatus (Stoelting) equipped with a rat adaptor. The primary advantage of chloral hydrate is the minimal cardiopulmonary depression seen at the normal doses. Meanwhile, chloral hydrate is easy to gain and shorter acting (1–2 h). 6-OHDA-HCL (32 ug dissolved in 8 μL of 0.9% normal saline containing 0.2% ascorbic acid) was injected into the right medial forebrain bundle (MFB) of rats at the same coordinates has been described before [16]. After three weeks the lesioned rats were tested behaviorally by an apomorphine hydrochloride-induced (0.5 mg/kg, intraperitoneal injection (i.p.)) rotation test and induction of LID models would select all animals performing at least 5 rotations/min contralateral orientation of the lesion site from the apomorphine treatment [17]. Once parkinsonism was stable, they were then treated with once-daily administration of L-dopa (25 mg/kg, i.p.) combined with benserazide (6.25 mg/kg, i.p.) for 3 weeks to induce a rat model of dyskinesia. The dose of L-dopa and benserazide were based on our group previous publications [15,16].

2.3. Drugs and treatment

PD rats were randomly divided into two groups and treated with vehicle or L-dopa/benserazide, respectively. Apomorphine hydrochloride (Sigma-Aldrich, USA) was administered (0.5 mg/kg). L-dopa (Sigma-Aldrich, 25 mg/kg) combined with benserazide-HCl (Sigma-Aldrich, 6.25 mg/kg) were given daily for 21 consecutive days. On day 22, pharmacological study with KN-93, a non-ATP competitive inhibitor of CaMKIIa, was dissolved in normal saline (KN-93-L group, 5 ug/kg; KN-93-H group, 10 ug/kg, respectively) and was infused 30 min prior to L-dopa intake into the striatal side ipsilateral to the 6-OHDA lesion with the stereotaxic coordinates of AP + 0.5 mm, ML + 2.5 mm, DV -4.6 mm [18]. Similarly, MK-801 (MK-801-L group, 0.1 mg/kg; MK-801-H group, 0.3 mg/kg, respectively), a non-ATP competitive inhibitor of NMDA was also infused into the same coordinates as KN-93. The control group of sham-lesioned rats was given saline for 21 days instead of L-dopa plus benserazide. Meanwhile, another group of sham-operated rats received intrastriatal administration of vehicle on day 22. The dose of KN-93 and MK-801 used was based on previous studies [11,19].

2.4. AIM ratings

A battery of axial, limb and orolingual movements (ALO AIM) ratings were used to assess the motor responses of PD rats treated with L-dopa at the day 7, 14, 21 and 22 as described elsewhere [15]. Briefly, for quantification of LID, rats were observed individually every 30 min from 30 to 120 min after the injection of L-dopa or saline. 0 = absent, 1 = present < 50% of the observation period, 2 = present > 50% of the observation time, 3 = present all the times but suppressible by external stimuli, and 4 = present all the times and not interfering by external stimuli [20]. For each rat, the maximum theoretical score per monitoring session was 4*3*4 = 48 regarded as ALO AIM scores.

2.5. Forelimb functional test (FFT) and apomorphine-induced rotation

In terms of parkinsonian disability score, a quantitative assessment of locomotor activity using forelimb functional test (FFT) was performed twice a week for 3 weeks, which was carried out 90 min after L-dopa administrated and was used as an index of parkinsonian disability score. The test was performed as our previous study [15]. All test processes were observed in a glass beaker with a diameter of 22 cm and a height of 35 cm to count bilateral forelimb use during vertical stand exploration. During a period of 60 min following L-dopa treatment, all weight-bearing wall contacts of the right and the left forepaws were counted every 20 min (3 min monitoring period for each). The final value was expressed as use of contralateral forelimb of the lesioned side with 6-OHDA compared with the total number of bilateral limb contacts [21,22]. Moreover, we used amphetamine-induced rotation test in a replicate study to verify that rats entering each treatment groups (L-dopa or L-dopa combined with ceftriaxone KN-93 or MK-801). *Apomorphine* (0.5 mg/kg, i.p.) test was also performed once a week for 3 weeks and rotations were quantified for 1 h following injection.

2.6. Western blot

After the last drug administration, all rats were sacrificed by 10% chloralic hydras (3.5 ml/kg,i.p.) intraperitoneal injection, and their brains were collected immediately on dry ice and two-tailed corpus striatums exhibited radial pattern were cut out in EP pipes. Corpus striatum tissues were homogenized in 20 mM Tris–HCl (pH 7.4), containing 1 mM NaF, 150 mM NaCl, 1% Triton-100 and freshly-added protease inhibitor cocktail, and 100 μM phenylmethylsulfonyl fluoride as previously described [23]. Then total proteins were separated on a 10% sodium dodecyl sulfate (SDS)–polyacrylamide gel and transferred overnight to polyvinylidene difluoride (PVDF) membranes in a Tris–-glycine transfer buffer. Then, the membrane was incubated with