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MPP+ inhibits mGluR1/5-mediated long-term depression in mouse hippocampus by calpain activation

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ABSTRACT

Neurotoxins are harmful to nervous system and cause either neuronal cell death or impairment of synaptic activity, which contributes to Parkinson's disease or other neuronal disorders. Hippocampal synaptic plasticity was proposed as a cellular model for memory processing. In this study, we reported a novel effect of neurotoxin, 1-methyl-4-phenylpyridinium (MPP+), on metabotropic glutamate receptor 1/5 agonist, 3,5-dihydroxyphenylglycine (DHPG)-induced hippocampal synaptic plasticity, and MPP+ incubation blocked DHPG-induced hippocampal long-term depression (LTD) in Schaffer collateral-CA1 synapses. Our further findings indicated that, this blockage was reversed by pre-application of calpain inhibitor III, but not by cathepsin inhibitors. Biochemical analysis showed that MPP+ treatment stimulated calpain activation, displayed by spectrin breakdown. Interestingly, the level and activity of protein tyrosine phosphatase 1B (PTP1B) were reduced after MPP+ incubation and the decrease of PTP1B was prohibited by calpain inhibitor III. In addition, PTP1B inhibitor also blocked DHPG-induced LTD, mimicking the effect of MPP+. In summary, our data implicated that MPP+ activated calpain-dependent PTP1B degradation, which subsequently impaired hippocampal LTD. This novel effect of MPP+ might partially explain the impairment of memory processing in the pathogenesis of PD.

1. Introduction

Genetic mutations or excessive environmental neurotoxin exposure contribute to the pathogenesis of Parkinson's disease (PD), which is usually symptomized by both of motor and non-motor dysfunctions (Terzioglu and Galter, 2008). Dopaminergic cell death is believed to be responsible for dopamine depletion and motor dysfunction (Michel et al., 2016). However, the mechanisms for non-motor dysfunctions, especially cognitive impairment or mood disorders in PD were not clarified. 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a neurotoxin precursor of 1-methyl-4-phenylpyridinium (MPP+), widely applied to produce PD model (Pifl et al., 2013). When MPTP is converted into MPP+ by the enzyme Monoamine oxidase B (MAO-B) and diffused in different brain area, it not only causes cell death in the substantia nigra, but also affects synaptic plasticity in the hippocampus (Huang et al., 2015).

Ionotropic glutamate receptors (Collingridge et al., 2009; Kennedy, 2016; Neves et al., 2008; Sweatt, 2016) and metabotropic glutamate (mGlu) receptors (Kim et al., 2007; Luscher and Huber, 2010; Mukherjee and Manahan-Vaughan, 2013; Sanderson et al., 2016; Xu et al., 2009) are critically required for both persistent forms of memory and synaptic plasticity. Acute application of mGlu receptor 1/5 agonist 3,5-dihydroxyphenylglycine (DHPG) stimulates a number of signaling pathways to sustain hippocampal long term depression (LTD) (Jakkamsetti et al., 2013; Mockett et al., 2011). Different from the function of N-methyl-D-aspartate (NMDA) receptors-dependent long-term potentiation (LTP) in spatial memory, DHPG-induced LTD is more related to memory extinction or reversal learning (Luscher and Huber, 2010; Michalon et al., 2012; Sanderson et al., 2016). As NMDA receptors-dependent synaptic plasticity and memory have been extensively investigated in the hippocampus of PD models (Costa et al., 2012; Zhu et al., 2011, 2012a, 2015a), it is critical to explore the effects of MPP+ on mGlu receptors-mediated synaptic plasticity.

As a Ca^{2+}-dependent protease, calpain has two major subtypes in the brain, calpain-1 and calpain-2, both functioning mainly through degrading the specific substrates (Zhu et al., 2015b). Recently, many substrates of calpain have been identified, including PH domain and Leucine rich repeat Protein Phosphatase (PHLPP), phosphatase and tensin homolog (PTEN), etc. (Baudry et al., 2015). Calpain has been reported to be over-activated to elicit cell death (De Simoni et al., 2013; Knaryan et al., 2014; Vosler et al., 2008). In addition to those functions, we previously demonstrated that calpain inhibitor blocked

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the effects of MPP^+ on depolarization-induced brain derived neurotrophic factor release (Zhu et al., 2015a).

We and others have also reported the effects of neurotoxins on memory and hippocampal synaptic plasticity in animal models (Costa et al., 2012; Zhu et al., 2011, 2012a, 2015a), NMDA receptors-dependent synaptic transmission and plasticity, as well as the memory consolidation were impaired in MPTP-induced PD model. Furthermore, previous studies also suggested that memory extinction was impaired in MPTP-induced PD mice (Kinoshita et al., 2015). To find the potential mechanisms, we investigated the effect of a low concentration of MPP^+ on DHPG-induced LTD in hippocampus and determined the roles of calpain activation performed in this process.

2. Materials and methods

2.1. Reagents

The information of the reagents used in this study were as following: calpain inhibitor III (10 μM, Calbiochem, USA); CA074 (1 μM, Tocris, USA), SID 26681509 (1 μM, Tocris, USA); MPP^+(25 μM, D048, Sigma, USA); (RS)-3,5-DHPG (100 μM, Tocris, USA); hyrtiosal (50 μM, Santa Cruz, USA). All other normal reagents without statement were from Sigma (USA).

2.2. Animals and preparation of hippocampal slices

Fifty male C57BL/6 mice (8-week old) were obtained from the Animal Center of Anhui Medical University (Hefei, China). Animal use and experimental protocols were approved by the animal care and use committee of Anhui University of Chinese Medicine.

After anesthesia by ether, mice brains were quickly removed following decapitation. The brains were transferred to oxygenated, ice-cold cutting medium including 124 mM NaCl, 26 mM $NaHCO_3$, 10 mM D-glucose, 3 mM KCl, 1.25 mM of KH_2PO_4, 5 mM $MgSO_4$, and 1.5 mM $CaCl_2$. Hippocampal transversal slices (350-μm thick) were prepared using a vibratome (Leica, Germany) and transferred to an interface recording chamber and exposed to a warm, humidified atmosphere with 95% O_2 and 5% CO_2 and continuously perfused with oxygenated and preheated (33 °C ± 0.5 °C) artificial cerebrospinal fluid (aCSF) (in mM) (110 NaCl, 5 KCl, 2.5 $CaCl_2$, 1.5 $MgSO_4$, 1.24 KH_2PO_4, 10 D-glucose, 27.4 $NaHCO_3$) at a flow speed of 1.6 ml/min.

After recovery, the slices were treated with 25 μM MPP^+, MPP^+ plus calpain inhibitor III, or MPP^+ plus cathepsin inhibitors for 30 min as indicated in the diagrams (Fig. 1A and Figs. 2A, B). After treatment, electrophysiological experiments were performed or the slices were collected in dry ice for biochemical experiments.

2.3. Electrophysiological experiments

After incubation for one hour in a recording chamber, a single glass pipette filled with 2 M NaCl was used to record field excitatory postsynaptic potentials (fEPSPs) elicited by stimulation of Schaffer collateral pathway with twisted nichrome wires (single bare wire diameter, 50 μm) placed in CA1 stratum radiatum. Responses were recorded by a differential amplifier (EXT-20F, npi electronic GmbH, Tamm, Germany) using 3 kHz high-pass and 0.1 Hz low-pass filters. LTD was induced by application of mGluR1/5 agonist- DHPG (100 μM, 10 min). Data were collected and digitized by Clampex and the slope of fEPSP was analyzed. LTD level was normalized to the baseline.

2.4. Biochemical experiments

Collected hippocampal slices including dentate gyrus after treatments were obtained and lysed. Protein concentrations were determined using the BCA protein assay kit (Thermo, US). Equivalent amounts of proteins were processed for sodium dodecyl sulphate-polyacrylamide gel electrophoresis and western blot as previously described (Zhu et al., 2015c). The primary antibodies used in this experiment were anti-Spectrin (1:1000, Millipore), anti-PTP1B (1:1000, Cell Signaling Technology) and anti-Actin (1:10,000, Abcam).

2.5. Protein tyrosine phosphatase 1B (PTP1B) activity

PTP1B activity was detected following the instructions of PTP1B activity assay kit (539,736, Millipore, USA). Briefly, the collected hippocampal slices were homogenated. Protein levels were determined using the BCA protein assay kit (Thermo, US). After treated with the substrate, colorimetric method was applied to detect PTP1B activity in the sample. To minimize systematic variance, PTP1B activity was normalized to the experimental control. Caspase-3 (ab39401, Abcam, USA) and protein phosphatase 2A (PP2A) (17–313, Millipore, USA) activities were also measured using the assay kits as previously described (Zhu et al., 2012b).

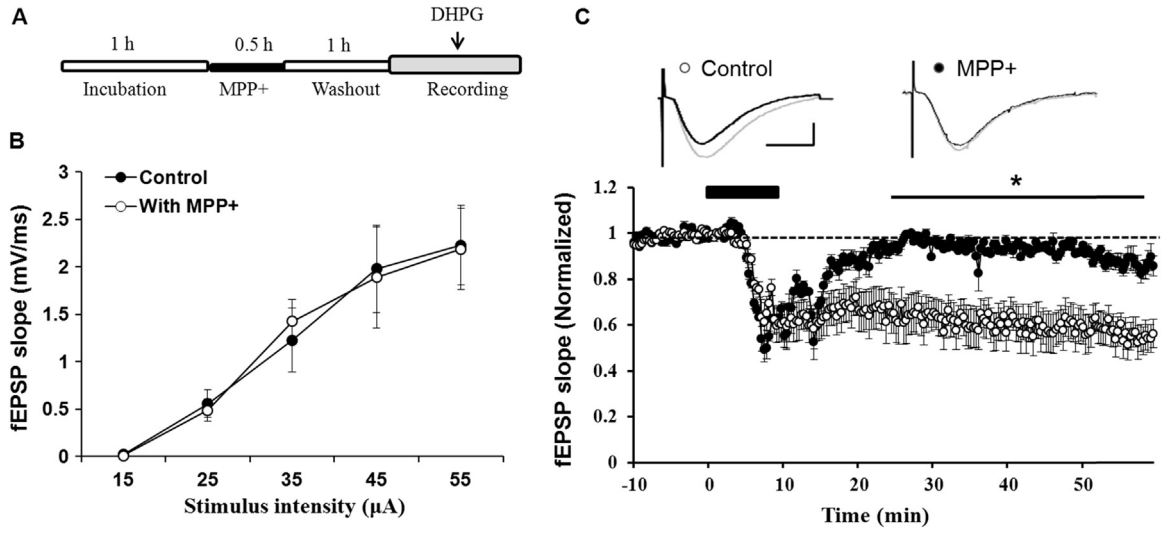

Fig. 1. MPP^+ blocks DHPG-induced LTD in hippocampus. A) The experimental procedure was illustrated. B) The input/output curves for pre- and 30 min post-MPP^+ application. The data were presented as means ± S.E.M. (n=12). C) DHPG-induced LTD in control (without MPP^+) and MPP^+-treated slices, means ± S.E.M. (n=5), black bar: DHPG application, *P < 0.05, compared with control. Black line indicates significant difference. The insertions represent analog traces for baseline (grey) and 50-min time point (black), scale bar: 10 ms/0.5 mV.