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Research Paper

Down-regulation of dorsal striatal αCaMKII causes striatum-related cognitive and synaptic disorders

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ABSTRACT

Alpha calcium/calmodulin dependent protein kinase II (αCaMKII) is a serine/threonine protein kinase which is expressed abundantly in dorsal striatum and is highly involved in the corticostriatal synaptic plasticity. Nevertheless, it currently remains unclear whether and how αCaMKII plays a in the striatum-related neural disorders. To address the above issue, lentivirus-mediated short hairpin RNA (shRNA) was used to silence the expression of αCaMKII gene in the dorsal striatum of mice. As a consequence of down-regulation of dorsal striatal αCaMKII expression, we observed defective motor skill learning in accelerating rotarod and response learning in water cross maze. Furthermore, impaired corticostriatal basal transmission and long-term potentiation (LTP), which correlated with the deficits in dorsal striatum-related cognition, were also detected in the αCaMKII-shRNA mice. Consistent with the above results, αCaMKII-shRNA mice exhibited a remarkable decline in GluA1-Ser831 and GluA1-Ser845 phosphorylation levels of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR), and a decline in the expression levels of *N*-methyl-D-aspartic acid receptor (NMDAR) subunits NR1, NR2A and NR2B. Taken together, αCaMKII down-regulation caused dorsal striatum-related cognitive disorders by inhibiting corticostriatal synaptic plasticity, which resulted from dysfunction of AMPARs and NMDARs. Our findings demonstrate for the first time an important role of αCaMKII in striatum-related neural disorders and provide further evidence for the proposition that corticostriatal LTP underlies aspects of dorsal striatum-related cognition.

1. Introduction

It has been widely recognized that the striatum plays an important role in learning of stimulus-response habits as well as motor, perceptual and cognitive skills (Graybiel and Grafton, 2015; Packard and Knowlton, 2002; Widmer et al., 2016; Yin et al., 2009). Dysfunction in striatum is associated with Parkinson's disease and Huntington's disease (Goldberg et al., 2005; Korzhova et al., 2014; Redgrave et al., 2010; Yang and Lu, 2011). Therefore, a deep understanding about the molecular and neuronal foundations underlying striatal functions would provide possible theoretical basis for these pathological disorders. The striatum receives glutamatergic innervations from the cerebral cortex (Parent and Hazrati, 1995). Moreover, cortico-striatal synaptic plasticity is widely accepted as the cellular mechanism underlying striatum-related cognitive functions (Barnes et al., 2005; Koralek et al., 2012; Yin et al., 2009). It has been reported that the predominant form of long term synaptic plasticity at the cortico-dorsomedial striatal (C-DMS) and cortico-dorsolateral striatal (C-DLS) synapses is long term potentiation (LTP) and long term depression (LTD), respectively (Yin et al., 2009). Especially, the *N*-methyl-D-aspartic acid receptor (NMDAR) is closely involved in the regulation of C-DMS LTP and certain forms of DMS related cognitive functions (Charpier and Deniau, 1997; Lovinger, 2010; Pisani et al., 2001). For instance, mice with pharmacological or genetic blockade of striatal NMDAR subunit showed severely impaired C-DMS LTP and lower performance in the tasks that require motor skill and flexible response sequences (Cory-Slechta et al., 1999; Dang et al., 2006; Lemay-Clermont et al., 2011).

Calcium/calmodulin-dependent protein kinase II (CaMKII), a downstream molecule of NMDAR signaling cascade, can be activated by the calcium elevation via the activated NMDARs. The interaction between NMDAR and phosphorylated CaMKII is important for LTP formation and memory in certain brain areas (Barcomb et al., 2016; Giese et al., 1998; Sanhueza et al., 2011). As one of the isoforms of CaMKII, alpha CaMKII (αCaMKII) was highly expressed in the dorsal striatum

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(Erondu and Kennedy, 1985). Picconi et al. (2004) proposed that motor abnormalities in experimental parkinsonism were accompanied with an abnormal activation of striatal αCaMKII. Besides, striatal αCaMKII gene expression was up-regulated after training on an accelerating rotarod (D'Amours et al., 2011). Taken together, these results indicate that αCaMKII should be involved in C-DMS LTP and striatum-related memory. However, few studies have addressed about how αCaMKII contributes to C-DMS synaptic plasticity as well as striatum-related cognitive functions until now.

Different from gene control, short hairpin RNA (shRNA) provides us an efficient tool to down-regulate selectively the expression of proteins in the specific brain region (Elbashir et al., 2001; Subramanian et al., 2017; Yu et al., 2002). Here, mice with dorsal-striatal αCaMKII specifically silenced by shRNA were used in our study. Our data demonstrate that αCaMKII deficiency impairs striatum-related learning, C-DMS LTP and the basal synaptic transmission, which are due to the dysfunction of NMDAR and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR). This sheds lights on the neural mechanisms of how the striatal αCaMKII is involved in striatum-related neural disorders for the first time.

2. Materials and methods

2.1. Mice

Experiments were performed on 2- to 3- month-old adult male C57BL/6 mice (25 g–30 g). The mice were housed in separate cages (n = 4 per cage) on a 12–12 h light/dark cycle (lights on at 7 a.m.) with constant ambient temperature (21 ± 1 °C)·Food and water were available ad libitum. All animal experiments described in this study were conducted according to Animals Act, 2006 (China) and approved by the Institutional Animal Care and Use Committee (IACUC approval ID #M07016) of the East China Normal University. Efforts were made to minimize animal suffering.

2.2. Administration of autocamtide2-related inhibitory peptide

Autocamtide2-related Inhibitory Peptide (AIP), an αCaMKII inhibitor, was obtained from Sigma, USA and dissolved at room temperature in 0.9% saline. Animals were anesthetized by intraperitoneal injection of sodium pentobarbital (40 mg/kg) and were places on a stereotaxic instrument. A stainless steel guide cannula was directly inserted into the dorsal striatum and fixed to the skull by dental acrylic. On the day of experiment, each side of brain received 2 μL of 1.6 μM AIP by using a stainless steel needle through guide cannula into the dorsal striatum. At the end of the experiment, the position of the cannulas was examined by histology (Supplemental Fig. 1).

2.3. Construction of lentivirus-αCaMKII-shRNA mice model

To probe the contribution of αCaMKII, mice were received a stereotaxic intrastriatal injection of 2 μL/side either a lentivirus-mediated short hairpin RNA (shRNA) (concentrated lentiviral stocks of 2×10^8) for silencing αCaMKII or non-functional shRNA with GFP as a control group. The sequences of shRNA targeting αCaMKII were (5′-CACCACCAUUGAGGACGAAdTdT-3′) (Chen et al., 2010). Stereotaxic injection was performed with a Hamilton syringe and at the following coordinates, calculated from bregma and skull surface: anterior, 0.6 mm; lateral, ± 1.5 mm; ventral, 2 mm. After the injection, mice were put back into home cages to recover for at least one week before experiments.

2.4. Behavior

2.4.1. Accelerating rotarod

To assess motor skill learning, we used the accelerating rotarod. One week after surgery, mice were placed on a rotarod apparatus and trained with ten trials per day for 8 consecutive days (Yin et al., 2009). The rod accelerated from 4 to 40 rpm in 300 s. After placing the mouse on the rod, each trial began. When the mouse fell off the rod or reached 300 s, each trial ended and latency was recorded. A resting time of 30 s was allowed between each trial. After 8 days of training, mice (average latency < 100 s) were eliminated from the test.

2.4.2. Water cross maze

Experiments were conducted in a cross maze. Four arms are made from the opaque Plexiglas (6 cm wide, 35 cm long and high for each arm). A removable and Plexiglas shield was used to block the entrance into the arm opposing to the starting position. Consequently, mice had to turn right or left. The maze was filled with white nontoxic liquid (23 °C) to obscure the hidden platform (1 cm below the liquid level). The maze were rotated every trial to reduce the contribution of spatial cues. Trials were started by placing mouse facing the wall of the tank in the start arm, which is randomly from north, south, west or east of the maze. Mice were trained to find the hidden platform in the arm always to the right of the start arm. As soon as the mouse made right turn to reach the platform (correct choice), it was allowed to stand on the platform for 15 s. Contrarily, if the mouse explored the arm without platform (incorrect choice), it was removed from the maze. Correct or incorrect choices were recorded. Mice were given 10 trials a day until they reached a learning criterion of > 9 correct choices for 3 consecutive days (Kheirbek et al., 2009).

2.4.3. Open field

Each mouse was placed in an acrylic open-field chamber (27 cm long × 27 cm wide × 38 cm high) for 15 min (CoulBourn Instrument). Illumination of open field was set to 16 lx. No background noise was provided. Infrared beams recorded the animal's location and path (locomotor activity). The amount of moving time and distance were measured using a Tru-scan DigBahv-locomotion Activity Video Analysis System (Coulbourn Instruments, USA) (Freitag et al., 2003).

2.4.4. Pole test

The mouse was placed head-upward on the top of a vertical rough-surfaced pole (diameter 0.8 cm; height 50 cm) and nesting material of the home cage was placed at the bottom of the pole. The time required for each mouse to turn around and descend the vertical pole was recorded. A maximum time of 120 s was allowed to execute the task. Each mouse was tested 5 times in succession with 10s rest (Chagniel et al., 2012; Freitag et al., 2003).

2.4.5. Stepping test

Stepping test was conducted on a flat table during the day light cycle. At the beginning, the animal was allowed to settle at one edge of the table, typically with all limbs on the table. The experimenter lifted the hind legs of each mouse by pulling up on the tail leaving only the forepaws touching the table. At a steady pace, the animal was pulled backwards for 50 cm. Each mouse was tested for 3 times. All tests were recorded by video, and the average number of adjusting steps from the forepaws was calculated (Blume et al., 2009; Heuer et al., 2012).

2.4.6. Wire suspension

A stainless wire (50 cm length, 0.2 cm diameter, elevated up to 37 cm from a surface) was fixed horizontally between two platforms. Each animal was hung on the middle of the wire and the latency to reach one of the platforms was recorded. As soon as the mice fell off or get to the edge of the wire, recording stopped. The maximal time allowed was set at 30 s. And the performance were scored according to the following system (Moran et al., 1995): 0, fell off; 1, hung onto the wire with two forepaws; 2, in addition to 1, attempted to climb onto the wire; 3, hung onto the wire with two forepaws and one or both hind paws; 4, hung onto the wire with all four paws with tail wrapped