Overexpression of βCaMKII impairs behavioral flexibility and NMDAR-dependent long-term depression in the dentate gyrus

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ABSTRACT

Behavioral flexibility is in close proximity to dentate gyrus (DG) function and long-term depression (LTD), but the role of DG LTD in behavioral flexibility has hitherto been unexplored. Although the functions of alpha-Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) have been studied extensively, the role of βCaMKII, a constituent of the CaMKII holoenzyme, in LTD and behavioral flexibility has not been investigated in vivo. Here using the βCaMKII-F90G transgenic (TG) mice, in which the inducible and reversible overexpression of βCaMKII is restricted to dentate gyrus (DG), we found that TG mice exhibited defective behavioral flexibility in two reversal tasks and seriously impaired *N*-methyl-D-aspartic acid receptor (NMDAR)-dependent LTD in DG medial perforant path (MPP). Consistent with the deficit in NMDAR-LTD, GluA1-Ser845, GluA1-Ser831 dephosphorylation and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) internalization were also disrupted during NMDAR-LTD in TG mice. Furthermore, these deficits were due to decreased activities of protein phosphatases (PP) 1/2A and glycogen synthesis kinase 3 beta (GSK3β), and overexpressed synaptic stargazin in TG mice. Importantly, all the deficits above could be reversed by 1-naphthylmethyl (NM)-PP1, a specific inhibitor of the exogenous βCaMKII-F90G. Taken together, our findings for the first time demonstrate that βCaMKII overexpression impairs behavioral flexibility and NMDAR-dependent LTD in DG MPP, which further confirms the close relationship between NMDAR-dependent LTD and behavioral flexibility.

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1. Introduction

Behavioral flexibility is a type of higher cognitive functions in mammals, which includes the inhibition of the old strategy acquired previously and the acquisition of a new strategy (Ragozzino,

2007). Behavioral flexibility reflects the ability of human or animals to adjust strategies and adapt to various external demands, which is intimately involved in their survival in novel or changeable environments. Furthermore, since patients or animal models of nervous system diseases, such as Alzheimer's Disease, Parkinson's disease, schizophrenia and depression, all exhibit impaired behavioral flexibility (Peterson et al., 2009; Darcet et al., 2014; Hitchcock et al., 2015; Granger et al., 2016; Reddy et al., 2016), a deeper understanding of the molecular and neural mechanisms underlying behavioral flexibility will be beneficial for development of therapeutic strategies.

Dentate gyrus (DG), as a major subregion of hippocampus, plays an important part in behavioral flexibility. It is reported that DG lesion (Xavier et al., 1999) or activity change of DG neurons (Garthe et al., 2009; Pan et al., 2012; Wang et al., 2012; Savanthrapadian et al., 2013) can both affect behavioral flexibility.

Recently more and more studies suggest that there is a link

between LTD and behavioral flexibility. LTD is considered crucial for weakening earlier memory traces and allowing the acquisition of new information at synapses (Nicholls et al., 2008). Inhibiting CA1 LTD pharmacologically can disrupt behavioral flexibility while enhancing CA1 LTD pharmacologically can facilitate behavioral flexibility (Duffy et al., 2007; Dong et al., 2013). These observations are well in line with some recent studies that have genetically attenuated CA1 LTD and observed diminished behavioral flexibility in the reversal learning phase of water maze and T-maze task (Kim et al., 2011; Mills et al., 2014; Lee et al., 2015). The results above confirm that CA1 LTD is involved in behavioral flexibility. Since DG is also an essential brain region for behavioral flexibility, it will be very significant to determine the role of DG LTD in behavioral flexibility.

Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), as a Ser/Thr protein kinase, plays multiple roles in cognitive functions and synaptic plasticity (Silva et al., 1992a,b; Mayford et al., 1995; Lisman et al., 2002; Cao et al., 2008). CaMKII is highly expressed in the central nervous system (Bennett et al., 1983), especially in hippocampus, where it makes up about 2% of total proteins (Erondu and Kennedy, 1985). There is a large quantity of CaMKII in synapses, especially in the dendritic spines (Feng et al., 2011; Ding et al., 2013). CaMKII has four isoforms: α, β, γ, δ, among which α and β are dominant in the nervous system. In general, α and β form CaMKII holoenzymes at a ratio of about 3:1 in the forebrain and 1:3−1:4 in the cerebellum (Miller and Kennedy, 1985). CaMKII can be activated by Ca^{2+}/CaM and becomes partially autonomous (Ca^{2+}-independent) upon autophosphorylation (αCaMKII at Thr286 and βCaMKII at Thr287) (Braun and Schulman, 1995; Colbran and Brown, 2004). Though CaMKII is traditionally considered to be mainly involved in LTP (Silva et al., 1992a,b; Frankland et al., 2001), recent studies have indicated a role of CaMKII in LTD. Inhibition of CaMKII activity can block NMDAR-dependent LTD (Coultrap et al., 2014). Moreover, transgenic mice with αCaMKII overexpressed show enhanced 3 Hz LTD in CA1 (Wang et al., 2003) but reduced LTD in Anterior cingulate cortex (ACC) (Wei et al., 2006).

Currently, studies on CaMKII focus mainly on αCaMKII isoform but not βCaMKII isoform. Constitutively, βCaMKII is highly homologous with αCaMKII except for its F-actin binding capacity, through which βCaMKII can regulate the dendritic patterning in the brain (Lin and Redmond, 2008; Sanabria et al., 2009; M. Kim et al., 2015a,b). Apart from the F-actin binding property, βCaMKII also differs from αCaMKII for its higher calcium sensitivity (Meyer et al., 1992; Brocke et al., 1999). βCaMKII homomers reach half-maximal autophosphorylation at approximately 15 nM calmodulin (CaM), whereas αCaMKII homomers require about 130 nM CaM (Brocke et al., 1999). In addition, the Ca^{2+}/CaM affinity of a heteromeric CaMKII holoenzyme increases with the enhancement of its β/α subunits ratio (Brocke et al., 1999; Nagasaki et al., 2014). Till now, there have been only a few reports about the role of βCaMKII in cognitive functions and synaptic plasticity. For example, βCaMKII knockout causes bidirectional impairment of synaptic plasticity in the parallel fiber-Purkinje cell pathway (van Woerden et al., 2009). Moreover, Cho et al. (2007) have shown that βCaMKII overexpression disrupts hippocampus-dependent memory consolidation and LTP in DG. However, it remains unknown whether and how βCaMKII plays a role in DG LTD and DG-related behavioral flexibility.

In order to address the above issues, we used βCaMKII-F90G transgenic (TG) mice, which were created using an inducible and reversible chemical genetic methods and expressed exogenous βCaMKII-F90G specifically in DG during 60−85 days postnatal. βCaMKII-F90G could be inhibited within 10 min through intraperitoneal injecting a rationally designed bulky inhibitor, 1-naphthylmethyl (NM)-PP1, without affecting the endogenous βCaMKII, thus suppressing the βCaMKII activity of transgenic mice down to the wild-type (WT) level (Cho et al., 2007). With electrophysiological, behavioral and molecular methods, we observed disrupted behavioral flexibility and NMDAR-LTD in the medial perforant pathway (MPP) of DG, accompanied by impaired α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) internalization and dephosphorylation in TG mice, which might be caused by the reduced activity of protein phosphatases (PP) and glycogen synthesis kinase 3 beta (GSK3β), and the increased synaptic stargazin.

2. Materials and methods

2.1. Animals

βCaMKII-F90G transgenic mice were provided by Dr. Tsien's lab (Cho et al., 2007). Through a silent mutation (i.e. replacing the Phe-90 with Gly in βCaMKII), mutant βCaMKII-F90G was generated and the ATP-binding pocket of βCaMKII-F90G kinase was enlarged. Notably, βCaMKII-F90G exhibited the kinase activities and ATP-binding affinities comparable to those of the native βCaMKII enzyme. NM-PP1 was designed to fit only this enlarged pocket (i.e. selectively block βCaMKII-F90G activity), but not the unmodified pocket of native βCaMKII (i.e. no effect on native βCaMKII activity). The βCaMKII-F90G transgene expression vector was constructed by inserting the 3.2-kb NotI fragment of the βCaMKII-F90G transgene into the unique NotI site of pMM279 containing an 8.5-kb αCaMKII promoter sequence. Using αCaMKII promoter-driven construct, we were able to selectively overexpress βCaMKII-F90G in forebrain. Interestingly, by *in situ* hybridization, Cho et al. (2007) observed that the expression of βCaMKII-F90G at the young adult phase (60−85 days postnatal) was spatially restricted to the dentate gyrus layer of the hippocampus. Acute intraperitoneal (i.p.) injection of 0.9% saline with 5 μM NM-PP1 (16.6 ng per gram of mouse body weight) into freely behaving transgenic mice could completely inhibit the enzymatic activity of βCaMKII-F90G within 10 min, consequently suppressing the total βCaMKII activity in transgenic mice down to the WT level, and this completely inhibiting effect could last for 35 min. In addition, chronic administration of 5 μM NM-PP1-containing water could also inhibit βCaMKII-F90G activity completely within 48 h, and the activity was restored to its pre-drug level within 24 h once the NM-PP1-containing water was removed. Similarly, bath application of 0.5 μM NM-PP1 in the brain slices could rapidly inhibit the βCaMKII-F90G activity while leaving native βCaMKII activity intact (Cho et al., 2007).

All the mice used in this research were 60−85 days male postnatal unless otherwise stated. The mice were housed 4 per plastic cage and maintained on a 12−12 h light/dark cycle (lights on at 7 a.m.), and given access to food and water ad libitum. All procedures were conducted according to Animals Act (2006) (China), and approved by the Institutional Animal Care and Use Committee (IACUC approval ID #M10020) of the East China Normal University. All efforts were made to minimize animal suffering, to reduce the number of animals used, and to utilize alternatives to in vivo techniques, if available.

2.2. Radial arm maze

The radial arm maze task was carried out in a plastic mouse-sized 8-arm radial arm maze with the length of 40 cm, the width of 7.5 cm, and the height of 13 cm. A plastic triangle was hung on the black curtain around the maze as a spatial cue for the mice. The protocol was designed mainly according to previous references (Clelland et al., 2009; Nakashiba et al., 2012) with some