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Short communication

CaMKII requirement for the persistence of in vivo hippocampal mossy fiber synaptic plasticity and structural reorganization

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ABSTRACT

CaMKII has been proposed as a molecular substrate for long-term memory storage due to its capacity to maintain an active autophosporylated state even after the decay of the external stimuli. The hippocampal mossy fiber-CA3 pathway (MF-CA3) is considered as a relevant area for acquisition and storage of different learning tasks. MF-CA3 pathway exhibits a form of LTP characterized by a slow initial increase in the EPSP slope that is independent of NMDA receptors activation. Our previous studies show that application of high frequency stimulation sufficient to elicit MF-CA3 LTP produces structural reorganization, in a manner independent of LTP induction, at the stratum oriens of hippocampal CA3 area 7 days after stimulation. However, the molecular mechanisms that underlie the maintenance of MF-CA3 LTP as well as the concomitant structural reorganization in this area remain to be elucidated. Here we show that acute microinfusion of myr-CaMKIINtide, a noncompetitive inhibitor of CaMKII, in the hippocampal CA3 area of adult rats during the late-phase of in vivo MF-CA3 LTP blocked its maintenance and prevented the accompanying morphological reorganization in CA3 area. These findings support the idea that CaMKII is a key molecular substrate for the long-term hippocampal synaptic plasticity maintenance.

1. Introduction

It is widely accepted that learning and memory rely on long-lasting activity dependent changes in synaptic efficiency. Long-term potentiation (LTP) is a long-lasting enhancement of synaptic strength and several studies have revealed that the long-lasting maintenance of LTP is accompanied by changes in the synaptic morphology that accompany the increment in synaptic efficiency (Lynch, Rex, & Gall, 2007). The hippocampal mossy fiber-CA3 pathway (MF-CA3) is considered as a relevant area for acquisition and storage of information. MF-CA3 LTP exhibits a form of LTP characterized by a slow initial increase in the EPSP slope that has been related to an independence of NMDA receptors activation (Nicoll & Schmitz, 2005). In previous studies, we had reported that application of high-frequency stimulation (HFS) sufficient to elicit LTP induced synaptogenesis, in a manner independent of LTP induction. Seven days after electrophysiological recordings, a band of Timm's staining, a frequently used marker to visualize MF buttons, revealed reorganization of mossy fiber connections at the stratum oriens (SO) of the CA3 area (Escobar, Barea, Derrick, Reyes, & Martinez, 1997; Gomez-Palacio-Schjetnan & Escobar, 2008; Ramos-Languren & Escobar, 2013; Schjetnan & Escobar, 2012). However, the molecular mechanisms that underlie the maintenance of long-lasting enhancement of synaptic transmission at MF-CA3 pathway remain to be elucidated.

It has been shown that calcium/calmodulin dependent protein kinase II (CaMKII) has an important role in LTP (Lisman, Yasuda, & Raghavachari, 2012). Impairment of CaMKII function through pharmacological (Malinow, Schulman, & Tsien, 1989) or genetic (Silva, Stevens, Tonegawa, & Wang, 1992) manipulations produce severe deficits in LTP. Activity-dependent increases in calcium concentration lead to CaMKII autophosphorylation. Since the increase in CaMKII autophosphorylation has been observed up to 8 h after stimulation (Ahmed & Frey, 2005) it has been proposed that CaMKII plays a central role not only in LTP induction, but also in the maintenance of synaptic potentiation (Lisman et al., 2012). In this regard, synaptic potentiation in hippocampal CA1 region is reverted when ant-CaMKIINtide, a noncompetitive inhibitor of CaMKII, is applied during the maintenance phase of CA1-LTP in vitro (Sanhueza, McIntyre, & Lisman, 2007; Sanhueza et al., 2011). The increase in synaptic activity also produces a translocation of CaMKII from the cytoplasm to the synapse (Otmakhov et al., 2004), where it interacts with several proteins such as NMDA receptors, densin-180, α-actinin and F-actin (Baucum, Shonesy,

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Rose, & Colbran, 2015; Bayer et al., 2006; Fink et al., 2003; Strack, McNeil, & Colbran, 2000; Walikonis et al., 2001), helping to sustain the activity dependent morphological changes implicated in memory maintenance (Jourdain, Fukunaga, & Muller, 2003).

Progress has been made in elucidating the contribution of CaMKII in the induction of LTP, but little is known about its role in the maintenance phase and in the sustained morphological changes accompanying the persistence of memories. Thus, in the present work we evaluated the role of CaMKII in the maintenance of in vivo MF-CA3 LTP, as well as, in the concomitant structural CA3 reorganization.

2. Methods

2.1. Animals

Thirty-nine male Wistar rats, weighing 360–390 g were used in the present study. They were housed individually under a 12/12-h light–dark cycle, with food and water available ad libitum and an average room temperature of 22 °C. Methods were carried out using adequate measures to minimize pain or discomfort in accordance with the guidelines of the Norma Oficial Mexicana and with the approval of the Local Animal Care Committee.

2.2. Electrophysiological procedure

Electrophysiological recordings at the MF pathway were performed in anesthetized animals as previously described (Ramos-Languren & Escobar, 2013; Schjetnan & Escobar, 2012; see Fig. 1A). Briefly, rats were anesthetized with pentobarbital (50 mg/kg i.p.), and given supplemental pentobarbital injections (10 mg/kg) at 1-h intervals to maintain a surgical level of anesthesia. Body temperature was maintained at 35 °C with a heating pad. Responses were recorded by using monopolar microinfusion electrodes placed in the *stratum lucidum* above the CA3 pyramidal layer of the dorsal hippocampus at the following stereotaxic coordinates: AP 2.9 mm, ML + 2.2 mm, DV 3.0 mm (Paxinos & Watson, 2007). Responses were evoked using a bipolar electrode via direct and unilateral stimulation of the MF at coordinates AP 3.5 mm, ML 2.0 mm, DV 3.1 mm (Paxinos & Watson, 2007; Fig. 1A). The evoked responses were stored for offline analysis using BrainWave software (DataWave, Broomfield, CO, USA). Low-frequency (0.05 Hz) responses were evoked for a 20-min baseline period, after which HFS (three trains/100 Hz/1 s/ 20 s intertrain interval) was applied and evoked responses were collected for 3 h. MF-LTP was calculated as a percentage change compared with the mean slope of the field potentials of the entire baseline period prior to HFS delivery. The metabotropic glutamate receptor II agonist 2-(2,3-dicarboxy-cyclopropyl) glycine (DCG-IV; Tocris, Ellisville, MO, USA; 0.5 μL of 0.5 μM in 5 min), was used at the end of the electrophysiological recordings to verify that the signal was generated by MF inputs (Calixto, Thiels, Klann, & Barrionuevo, 2003).

2.3. Experimental design

Animals were divided into the following groups: (1) HFS group (n = 7), which had electrodes implanted and received HFS capable of inducing LTP; (2) HFS + Ntide group (n = 7), which in the same conditions of HFS group received intrahippocampal CA3 microinfusion of the high affinity CaMKII noncompetitive inhibitor myr-CaMKIINtide, which only binds to the activated conformation of CaMKII (5 μM/μl ACSF/0.02 μl/mim; Gomez-Monterrey et al., 2013; Sanhueza et al., 2007) prepared with artificial cerebrospinal fluid (ACSF) as vehicle two hours after HFS delivery; (3) HFS + ACSF group (n = 7) which under the same conditions as the HFS + Ntide group, received intrahippocampal microinfusion of ACSF (1 μL); (4) BL + Ntide group (n = 7) which had electrodes implanted and without prior manipulation received intrahippocampal microinfusion of myr-CaMKIINtide (5 μM/μl ACSF/0.02 μl/min); (5) intact control group (CON, n = 5), that was used for morphological analysis.

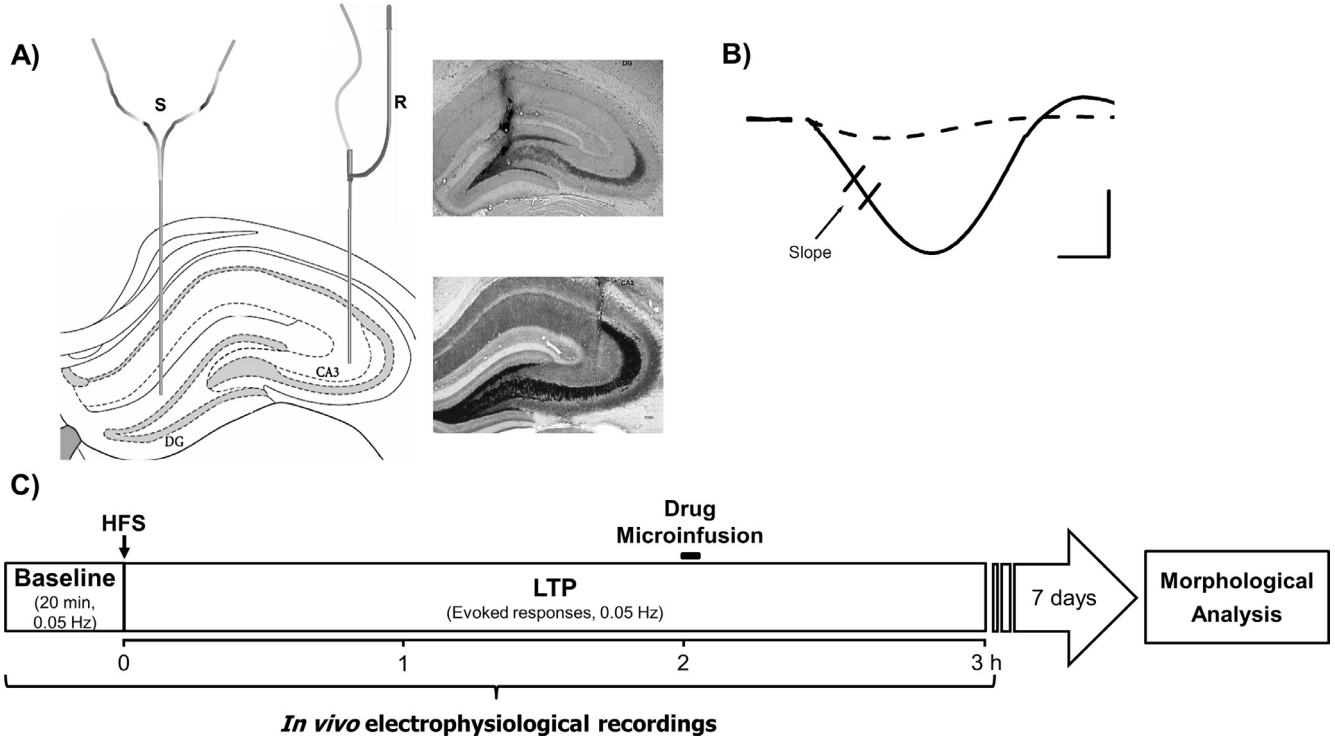

Fig. 1. (A) Schematic representation and representative images of electrode placement showing the stimulated (S) and recorded (R) sites in a coronal plane. Scale bar: 1 mm. DG: Dentate Gyrus. (B) Representative traces of the EPSP showing the component for slope measures, obtained at baseline (full line) and after DCG-IV application (dotted line). Note that DCG-IV selectively blocked MF responses. Scale bar: 5 ms and 0.25 mV. (C) Schematic representation of the experimental procedure.