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Rapid communication

Assessing the roles of presynaptic ryanodine receptors and adenosine receptors in caffeine-induced enhancement of hippocampal mossy fiber transmission

Ikuma Sato*, Haruyuki Kamiya

Department of Neurobiology, Hokkaido University School of Medicine, Kita 15 Nishi 7, Kita-ku, Sapporo, 060-8638, Japan

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ABSTRACT

Caffeine robustly enhances transmitter release from the hippocampal mossy fiber terminals, although it remains uncertain whether calcium mobilization through presynaptic ryanodine receptors mediates this enhancement. In this study, we adopted a selective adenosine A1 blocker to assess relative contribution of A1 receptors and ryanodine receptors in caffeine-induced synaptic enhancement. Application of caffeine further enhanced transmission at the hippocampal mossy fiber synapse even after full blockade of adenosine A1 receptors. This result suggests that caffeine enhances mossy fiber synaptic transmission by two distinct presynaptic mechanisms, i.e., removal of A1 receptor-mediated tonic inhibition and ryanodine receptor-mediated calcium release from intracellular stores.

Calcium ions (Ca^{2+}) play important roles in neurotransmitter release from presynaptic terminals and its modulation by neuronal activity. In addition to Ca^{2+} influx from extracellular fluid, Ca^{2+}-induced Ca^{2+} release (CICR) from intracellular stores regulate presynaptic intracellular Ca^{2+} concentration in some types of synapses. The roles of presynaptic Ca^{2+} store in regulating transmitter release have been suggested for a long time (Onodera, 1973; Erulkar and Rahamimoff, 1978). Recently, much evidence has been accumulated for the presence of ryanodine-sensitive store in presynaptic terminals and/or axons at the hippocampal mossy fiber synapse onto CA3 pyramidal cell (Liang et al., 2002; Lauri et al., 2003; Sharma and Vijayaraghavan, 2003). This notion was also supported by the finding that application of caffeine, drug acting on ryanodine receptor channels to release Ca^{2+} from intracellular stores, strongly enhanced synaptic transmission at the hippocampal mossy fiber synapse (Shimizu et al., 2008). This result also suggests the presence of functional ryanodine receptors and possible CICR mechanisms may be involved in robust activity-dependent presynaptic plasticity characteristic for this synapse.

However, caffeine is also known to block adenosine A1 receptors potently (Fredholm et al., 1999). It was found that extracellular adenosine acts on presynaptic A1 receptors and suppresses transmitter release via activation of $G_{i/o}$ (Dunwiddie and Hoffer, 1980). Previous studies demonstrated that application of the selective A1 receptor antagonist induced potent enhancement of mossy fiber response in vitro (Moore et al., 2003) and also *in vivo* (Klausnitzer and Manahan Vaughan, 2008; Hagena and Manahan Vaughan, 2010) by reducing tonic inhibitory effects of ambient adenosine. This effect is prominent at the mossy fiber synapse in comparison with adjacent associational–commissural synapse, and contributes, at least partly, to its characteristics such as low-release probability, large frequency facilitation and paired-pulse facilitation (Nicoll and Schmitz, 2005).

Therefore, it remains to be determined whether activation of ryanodine receptors, in addition to blocking A1 receptors, mediates the caffeine-induced enhancement at this synapse. In this study, we addressed this issue using a selective blocker of A1 receptor (8-cyclopentyl-1,3-dipropylxanthine; DPCPX) and an inhibitor of ryanodine receptor channels (ryanodine). We found that caffeine enhanced the hippocampal mossy fiber response even under full blockade of A1 receptors, and that the magnitude of enhancement was decreased in the presence of ryanodine. We also measured presynaptic Ca^{2+} levels within mossy fiber terminals by fluorescence recording during application of DPCPX and caffeine. A part of this work was presented elsewhere in an abstract form (Sato and Kamiya, 2009).

All experiments were performed according to the guidelines for the care and use of laboratory animals of Hokkaido

* Corresponding author. Tel.: +81 11 706 5028; fax: +81 11 706 7863.
E-mail address: ikuma_sato@ec.hokudai.ac.jp (I. Sato).

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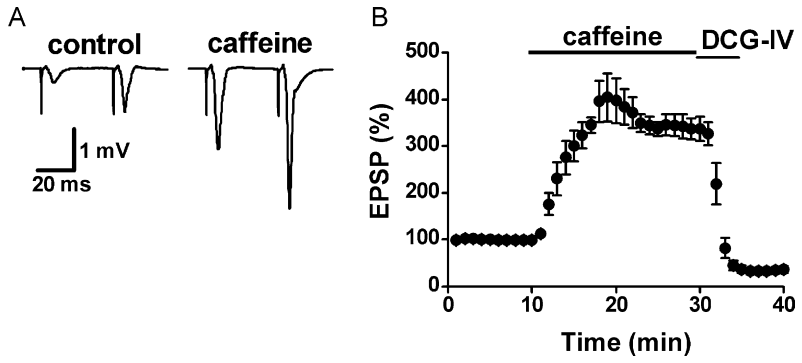

Fig. 1. Effects of caffeine on synaptic transmission at the mossy fiber-CA3 synapse. (A) The representative traces recorded before and after application of 10 mM caffeine. It should be noted that paired-pulse facilitation reduced during application of caffeine. (B) Time course of the amplitude of the first EPSPs. At the end of each experiment, 1 μM DCG-IV was applied to confirm that the mossy fiber was selectively stimulated. Closed circles and error bars represent mean and SEM of multiple experiments, respectively. Six trials are included in each data point. The stimulus interval is set for 10 s.

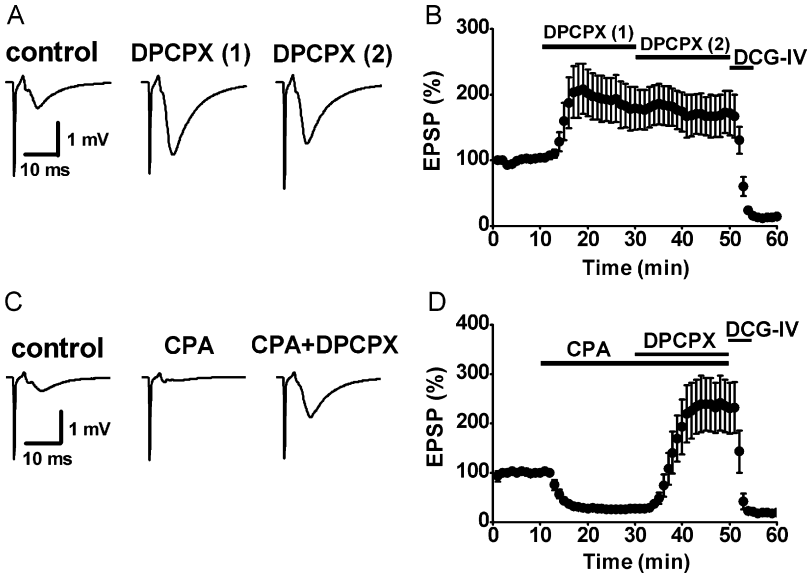

Fig. 2. Effects of selective A1 receptor antagonist DPCPX. (A) The representative traces recorded during consecutive application of DPCPX at 1 μM and 2 μM, respectively. (B) Time course of the first EPSPs. (C and D) Antagonism of adenosine A1 receptor-mediated synaptic inhibition by DPCPX. DPCPX (at 1 μM) completely reversed the inhibition of mossy fiber fEPSPs by 800 nM CPA, a selective agonist of A1 receptors. Note that DPCPX further enhanced the fEPSPs than those before application of CPA and reached to the level almost identical with application of DPCPX alone.

University. Recordings of field excitatory postsynaptic potentials (fEPSPs) elicited by mossy fiber stimulation were made as described before (Kamiya et al., 1996). Briefly, transverse hippocampal slices (400 μm thick) were prepared from C57BL/6J mice (12–28 days old) in an ice-cold sucrose solution containing (in mM) 40 NaCl, 2.5 $NaHCO_3$, 10 glucose, 150 sucrose, 4 KCl, 1.25 NaH_2PO_4, 0.5 $CaCl_2$, and 7 $MgCl_2$ (Geiger et al., 2002). Slices were continuously superfused with a solution (artificial cerebrospinal fluid; ACSF) containing (in mM) 127 NaCl, 1.5 KCl, 1.2 KH_2PO_4, 26 $NaHCO_3$, 10 glucose, 4 $CaCl_2$, and 4 $MgCl_2$, which was saturated with 95% O_2 and 5% CO_2.

Mossy fibers were stimulated at the granule cell layer of dentate gyrus through a concentric bipolar electrode of 100 μm tip diameter, and the evoked fEPSPs were recorded in the stratum lucidum of the CA3 region through a glass microelectrode of about 10 μm tip diameter filled with ACSF. Group II metabotropic glutamate receptor agonist (2 S,2′R,3′R)-2-(2′,3′-dicarboxycyclopropyl)glycine (DCG-IV, 1 μM) (Tocris Bioscience, Bristol, UK) was applied at the end of each experiment to verify the mossy fiber origin of the fEPSPs.

In this study, we paid much attention to reduce accumulation of DPCPX (Tocris Bioscience, Bristol, UK) in the perfusing system. Several previous studies have pointed out that DPCPX is readily accumulated in the perfusing system due to its poor water solubility (McPherson et al., 2010) and thereafter released into perfusing solution gradually. This unusual property of DPCPX will result in underestimation of action of adenosine A1 receptors. Therefore we washed the system thoroughly with 70% ethanol at least for 20 min before and after each experiment.

Fluorescence recordings of presynaptic Ca^{2+} at mossy fiber terminals were made as described before (Kamiya and Ozawa, 1999). In brief, membrane permeable Ca^{2+} indicator Oregon Green 488 BAPTA-1 AM (OGB1, 0.1 mM in DMSO with 1% pluronic acid) (Invitrogen, Carsbad, CA) was locally injected into stratum lucidum and loaded into mossy fiber terminals via axons. We attempted to detect minimal changes of basal fluorescence levels by using high-affinity Ca^{2+} indicator OGB1. The fluorescence was measured every 5 min with a single photodiode. The fEPSPs were also recorded simultaneously as described above.

All data are expressed as mean ± SEM. For statistical analysis, we adopted peak values of fEPSPs after application of each drug, and they were presented in percentile form. Statistical analysis was performed using Student's *t*-test, and *P* values less than 0.05 were accepted for statistical significance.