Cholinergic modulation for hippocampal long-term potentiation

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Abstract Long-lasting change of the synaptic efficacy of neurotransmission in the hippocampus is thought to be one of the physiological bases of memory. Acetylcholine, one of the neurotransmitters, is mainly released from the medial septum to the hippocampus. The activation of muscarinic acetylcholine receptors (mAChR) reportedly enhances hippocampal synaptic plasticity; however, the mechanism by which it does this remains unclear. We addressed the involvement of the inhibition of Kv7/M K⁺ channels, which are a target of mAChR modulation, during mAChR activation-induced enhancement of long-term potentiation (LTP) at rat hippocampal Schaffer collateral (SC)-CA1 synapses.

Key words : acetylcholine, potassium channel, hippocampal plasticity

Hippocampal synaptic plasticity has been considered to be one of the physiological bases of memory (Bliss & Collingridge, 1993). This plasticity is mainly mediated by glutamate, the main excitatory neurotransmitter in the hippocampus. At Schaffer collateral (SC)-CA1 synapses, AMPA-type glutamate receptors (AMPA receptors) receive presynaptically released glutamate. When postsynaptic membranes are strongly activated (depolarized) by an intense stimulus, such as high-frequency stimulation (e.g. tetanic stimulation), NMDA-type glutamate receptors (NMDA receptors) are activated, which results in the influx of Ca²⁺. The influx of Ca²⁺ activates intracellular second messengers (e.g. CaMKII), and then the number of AMPA receptors at the membrane surface increases and they become phosphorylated. After that, the response to the released glutamate is enhanced due to the changes of the AMPA receptors. This long-lasting enhancement of

the efficacy of neurotransmission is called long-term potentiation (LTP).

In the hippocampus, acetylcholine, one of the neurotransmitters, is mainly released from the fibers originating from the medial septum. It has been reported that acetylcholine-related enzymes are reduced in Alzheimer's disease patients (Davies & Maloney, 1976; Geula & Mesulam, 1996). Furthermore, agonists and antagonists of acetylcholine receptors affect memory function, that is, they lead to the improvement and impairment of memory, respectively (Fitz, Gibbs, & Johnson, 2008; Gold, 2003). Thus, the cholinergic system is also a research target of the cellular mechanism of memory functions. There are two types of acetylcholine receptor: muscarinic acetylcholine receptors (mAChR) and nicotinic acetylcholine receptors (nAChR). The activation of mAChR reportedly enhances the magnitude of LTP at SC-CA1 synapses *in vivo* and *in vitro* (Ovsepien, Anwyl, & Rowan, 2004; Shinoue, Matsui, Taketo, & Manabe, 2005); however, the mechanism by which it enhances synaptic plasticity remains unclear.

The inhibition of K⁺ currents is one candidate because mAChR activation is known to inhibit some K⁺ currents (Brown & Adams, 1980; Higashida, Hoshi, Zhang,

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Yokoyama, Hashii, Jin, Noda, & Robbins, 2005). The inhibition of K^+ channels can induce LTP. For example, the application of a blocker of K^+ channels, tetraethylammonium (TEA), induces LTP in all hippocampal subregions *in vitro* (Aniksztejn & Ben-Ari, 1991; Coogan, O'Leary, & O'Connor, 1999; Suzuki & Okada, 2007). This form of LTP is independent of the activation of NMDA receptors, but dependent on voltage-dependent Ca^{2+} channels (VDCC) instead.

The influx of Ca^{2+} via VDCC is a trigger for the induction of LTP as described above.

Recently, it was reported that the activation of mAChR enhanced NMDA receptor-mediated current via the inhibition of small conductance K^+ channels (SK channels) (Buchanan, Petrovic, Chamberlain, Marrion, & Mellor, 2010). The enhancement of NMDA receptor-mediated current resulted in the enhancement of LTP. Another candidate is Kv7/M K^+ channels because the

Figure 1. The enhancement of LTP by cholinergic activation.

A: Schema of hippocampal slice and electrodes. Schaffer collaterals (SC) were stimulated every 10 s (Stim.) and field excitatory postsynaptic potentials (fEPSPs) were recorded from the CA1 stratum radiatum (Rec.). The 40-Hz stimulation of the stratum oriens (str. ori. stim.) was applied 30 s before the tetanic stimulation of SC, when needed. B: Tetanic stimulation (arrow) of SC induced typical LTP (open circles). When a 40 Hz stimulus was applied to the stratum oriens, the magnitude of the LTP was enhanced (filled circles) ($p < 0.01$, compared with control). Bath application of the mAChR antagonist atropine (10 μ M) inhibited the enhancement of LTP by the stimulation of the stratum oriens (gray squares) ($p < 0.01$, compared with str. ori. stim.). This atropine-induced inhibition was rescued by the co-application of the Kv7/M K^+ channel blocker XE-991 (10 μ M) (gray circles) ($p < 0.01$, compared with control). The white bar indicates the timing of drug application. Insets: Typical waveforms of the synaptic responses obtained before (1) and 60 min after (2) the tetanic stimulation under each condition. The relative amplitudes of the fEPSP are shown as the mean $\pm$ S.E. The traces were taken at the times indicated by the numbers in the figure. Scale: 5 ms, 0.5 mV. ** $p < 0.01$, * $p < 0.05$.

activation of M1-type mAChR (M1 mAChR) has been shown to inhibit Kv7/M K⁺ channels (Delmas & Brown, 2005; Higashida, Hoshi, Zhang, et al., 2005), and M1 mAChR is mainly involved in the facilitating effect of mAChR on LTP (Shinoe, Matsui, Taketo, et al., 2005). Thus, we electrophysiologically examined whether Kv7/M K⁺ channels are involved in the facilitating effect of mAChR activation on tetanus-induced LTP in the rat hippocampal CA1 area (Suzuki & Okada, 2012). This research is summarized in this paper.

Repetitive stimulation (40 Hz, 0.5 s) to the stratum oriens in CA1 was applied 30 s before the tetanic stimulation of the SC. The stratum oriens contains fibers from the medial septum. The magnitude of tetanus-induced LTP was enhanced when the stratum oriens was stimulated (Figure 1, filled circle). The enhancement of tetanus-induced LTP was blocked in the presence of the mAChR antagonist atropine (Figure 1, gray square). When inhibitor of the Kv7/M K⁺ channel, XE-991, was applied, the enhancement of tetanus-induced LTP was observed, even in the presence of atropine (Figure 1, gray circle). Furthermore, the application of Kv7/M K⁺ channel inhibitor enhanced tetanus-induced LTP without stimulation of the stratum oriens (Figure 2, XE-991 w/o str. ori. stim.). When the stimulation of the stratum oriens was applied in the presence of XE-991, the magnitude of tetanus-induced LTP was not additionally enhanced. These results suggest that the activation of mAChR and the inhibition of the Kv7/M K⁺ channel are involved in the enhancement of tetanus-induced LTP by the stimulation of the stratum oriens.

What is the intracellular mechanism behind the enhancement of tetanus-induced LTP by the activation of mAChR? The activation of mAChR activates an intracellular signaling cascade. One of the targets is phospholipase C (PLC). The activation of PLC makes diacylglycerol (DAG) and IP₃ from PIP₂. DAG activates the A-kinase anchoring protein (AKAP)-protein kinase C (PKC)

complex, which results in inhibition of the Kv7/M K⁺ channel. When PLC was inhibited by a selective blocker, U-73122, mAChR activation-induced enhancement of tetanus-induced LTP was blocked (Figure 2, U-73122). Thus, the activation of PLC is important for the enhancement of tetanus-induced LTP. As described above, while the influx of Ca²⁺ via NMDA receptors is crucial for LTP induction, tetanic stimulation with preceding stimulation of the stratum oriens induced

Figure 2. The magnitude of LTP in each experimental condition.

The magnitude of LTP 50-60 min after the tetanic stimulation was calculated. In control and XE-991 w/o str. ori. stim. conditions, tetanic stimulation was applied to the SC without str. ori. stim. In other conditions (str. ori. stim., Atropine, Atropine + XE-991, U-73122, D-AP5 and nickel), the stimulation of the stratum oriens was applied before the tetanic stimulation. The application of atropine inhibited the enhancement of tetanus-induced LTP by str. ori. stim. (Atropine). However, the application of Kv7/M K⁺ channel inhibitor, XE-991, enhanced the magnitude of tetanus-induced LTP even in the presence of atropine or without str. ori. stim. (Atropine + XE-991 and XE-991 w/o str. ori. stim., respectively). On the other hand, intracellular PLC blocker U-73122 and T/R-type voltage-dependent Ca²⁺ channel blocker nickel inhibited the enhancement of tetanus-induced LTP. Moreover, str. ori. stim. with tetanic stimulation induced LTP under the blockade of NMDA receptors (D-AP5). The magnitudes of LTP are shown as the mean $\pm$ S.E. The statistical significances were calculated compared with str. ori. stim. **p < 0.01.

Figure 3. The proposed schema of the enhancement of tetanus-induced LTP by cholinergic modulation.

① The stimulation of cholinergic input releases acetylcholine, and then muscarinic acetylcholine receptors (mAChRs) are activated. mAChR activates an intracellular signaling pathway that includes the phospholipase C (PLC) cascade. ② The activation of PLC makes diacylglycerol (DAG) and IP3. DAG activates protein kinase C (PKC) and Kv7/M K⁺ channels are inhibited. ③ The inhibition of Kv7/M K⁺ channels results in the depolarization of cell membrane, and then voltage-dependent calcium channels are activated, which causes an influx of Ca²⁺.

LTP in the presence of NMDA receptor antagonist, D-AP5 (Figure 2, D-AP5). That is, the intracellular concentration of Ca²⁺ is increased by another mechanism. Because the influx of Ca²⁺ via VDCC is important for LTP induction by the K⁺ channel blocker TEA, it is thought that the depolarization of the cell membrane by the inhibition of the Kv7/M K⁺ channel might activate VDCC. Consistent with this hypothesis, the blockade of T/R-type VDCC prevented the mAChR activation-induced enhancement of tetanus-induced LTP (Figure 2, nickel). Taking these findings together, the stimulation of cholinergic input releases acetylcholine, and then mAChR is activated. mAChR activates an intracellular signaling pathway that includes the PLC cascade, and Kv7/M K⁺ channels are inhibited. The inhibition of Kv7/M K⁺ channels results in depolarization of the cell membrane, and then VDCC is activated, which causes an influx of Ca²⁺ (Figure 3).

It is reported that the concentration of acetylcholine in the hippocampus is enhanced during the acquisition of operant behavior (Orsetti, Casamenti, & Pepeu, 1996) and novel stimuli enhance acetylcholine release in the hippocampus (Acquas, Wilson, & Fibiger, 1996). Therefore, the enhancement effect of LTP by mAChR activation might be important for memory acquisition.

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