論文内容の要旨

Neuromodulation of Excitatory Synapses in the Lateral Nucleus of the Amygdala

扁桃体外側核における興奮性シナプスの神経調節

指導教員　真鍋 俊也 教授
東京大学大学院 医学系研究科
平成 19 年 4 月入学　博士課程
脳神経医学専攻

田附 千絵子

Fear is exhibited by all mammals, and appears to be part of a universal survival strategy. It is known that the amygdala is the center for the acquisition, storage, and expression of fear memory (LeDoux, 2000). The lateral (LA) nucleus of the amygdala receives a pair of sensory information of conditioned (tone) and unconditioned (electric shock) stimuli, and the association of these coincident inputs is considered to be stored as long-term potentiation (LTP) at thalamo-LA synapses. Emotionally arousing experiences recruit hormone secretion and excite neuromodulatory systems, whereby enhances response to fear or fear conditioning (Rodrigues, 2009). In fact, behavioral studies have shown that cholinergic or catecholaminergic activation enhances amygdala-dependent aversive experience (IntroiniCollison, 1996; McGaugh, 2004). However, reported effects of

neuromodulators on amygdalar synapses are diverse and not yet conclusive (reviewed in Pape, 2010). Therefore, it is important to examine the effects and mechanisms of neuromodulation on basal synaptic transmission in LA principal neurons to promote the understanding of how emotional arousal or attention influences fear-related behaviors.

Using coronal slices containing the amygdala of C57BL/6J male mice (Fig. 1), I recorded excitatory postsynaptic currents (EPSCs) from principal neurons in the dorsal subdivision of the LA by stimulating thalamic afferent fibers. Whole-cell voltage-clamp recordings were made at -80 mV in the presence of picrotoxin in order to ensure excitatory currents. First, I found that application of cholinergic agonist carbachol (CCh) induced transient suppression of the amplitudes of evoked EPSCs while increasing paired pulse ratio (PPR) (Fig. 2). Furthermore, miniature EPSC frequencies decreased in the presence of CCh (Fig. 3). These results strongly suggested that CCh acts mainly on presynaptic terminals and suppresses vesicle release. I next examined which type of acetylcholine receptors (AChRs) was responsible for this suppression and whether the site of action was pre- or postsynaptic. Pharmacological manipulation and genetic ablation of muscarinic AChRs revealed that CCh directly acted on muscarnic M_4 receptors on the presynaptic terminals (Fig. 4, 5). In addition, N-type voltage-dependent calcium channel (VDCC) was a target inhibited by M_4 receptor downstream signaling

(Fig. 6). CCh-induced suppression of EPSCs remaining in the presence of N-type VDCC blocker could be due to direct inhibition of vesicle release machinery as shown in hippocampus where muscarinic activation of $G_{i/o}\alpha$ inhibited PKA-dependent phosphorylation of SNARE proteins and thereby modulated release machinery (Chheda, 2001). Taken together, cholinergic activation might have two distinct sites of action: vesicle release machinery and N-type VDCC (Fig. 10A).

Next, I examined the effects of norepinephrine (NE) and dopamine (DA) on basal synaptic transmission at thalamo-LA synapses. I found that NE with affinity for both αAR and βAR suppressed AMPAR-mediated EPSCs as well as NMDAR-mediated EPSCs and enhanced PPR (Fig. 7), suggesting that NE was a negative modulator presumably by suppressing presynaptic release. In contrast, activation of βAR with isoproterenol increased AMPA-EPSC amplitudes without changing PPR (Fig. 8). These results suggested that presynaptic αAR played a predominant role in adrenergic suppression under physiological conditions, and also demonstrated that αAR and βAR had opposing roles in excitatory transmission at thalamo-LA synapses. Finally, I found that DA also suppressed excitatory synaptic transmission (Fig. 9). Intriguingly, receptors at presynaptic terminals for the neuromodulators I examined are M_2 and M_4 receptors, α_2ARs, dopamine D_2 receptors,

all of which are coupled to $G_{i/o}$ proteins, implying the existence of a common mechanism for presynaptic suppression (Fig. 10B).

My finding that neuromodulators induced suppression of excitatory neurotransmission between thalamus and LA principal neurons seems to be contradictory to a number of previous reports that suggested the enhancement of LTP induction by neuromodulators. Knockout mice of M_2 receptor demonstrated stronger disinhibition of GABAergic than glutamatergic transmission (Seeger, 2004). The application of DA or NE gated LTP induction at thalamo-LA synapses by suppressing GABAergic inputs (Bissiere, 2003; Tully, 2007). Thus, suppression by neuromodulators of presynaptic function and excitatory transmission at thalamo-LA connections may be outweighed by concomitant disinhibition of inhibitory neurons allowing the enhancing LTP induction.