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学位授与の要件	学位規則第４条第１項該当 医学系研究科未来医療開発専攻
学位論文名	Roles of endocannabinoids in heterosynaptic long-term depression of excitatory synaptic transmission in visual cortex of young mice （幼若マウス視覚野興奮性シナプス伝達の異シナプス性長期抑圧における内因性カンナビノイドの役割）
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論文内容の要旨

〔Purpose〕

In the developing visual cortex, long-term potentiation (LTP) and long-term depression (LTD) of synaptic transmission are supposed to play a role in experience-dependent changes in function of cortical neuronal circuits. Tetanic stimulation of one of two afferent pathways converging to neurons in the visual cortex induces two types of changes, homosynaptic LTP (homo-LTP) in the activated pathway and heterosynaptic LTD (hetero-LTD) in the non-activated pathway under a certain condition. The latter form of synaptic plasticity was not systematically investigated in previous studies, whereas homosynaptic LTD has been extensively studied. The purpose of this research is to determine whether hetero-LTD is induced in visual cortical slices of mice and if so, what mechanism underlies this form of LTD.

〔Methods〕

1. Acute slices of the visual cortex from wild-type mice (C57BL/6), aged from 14 to 20 postnatal days (P14-20) were used. In the experiments in which the age dependence of hetero-LTD was tested, the same line of mice at P7-10 and P35-41 were also used.

2. Two stimulating electrodes were placed at separate sites in layer IV of cortical slices. Whole-cell recordings from pyramidal neurons in layer II/III of the visual cortex were carried out under infrared differential interference contrast optics. Excitatory postsynaptic potentials (EPSPs) evoked by test stimulation of layer IV at 0.05 Hz were recorded in the current-clamp mode.

3. To induce homo-LTP and hetero-LTD, theta-burst stimulation (TBS) paired with postsynaptic depolarization at 0 mV for 40 s was applied to one site of layer IV. TBS consisted of 4 trains at 0.1 Hz, each train consisting of 10 bursts at 5 Hz, and each burst consisting of 4 pulses at 100 Hz. The intensity of each pulse was twice the test pulse intensity.

4. The drugs used in the present study included a Ca^{2+} chelator (BAPTA) and various antagonists and agonists for glutamate receptors and the type 1 of endocannabinoid (eCB) receptors. These drugs were applied either through the perfusion medium or the internal solution of recording pipettes. When drugs were applied through the internal solution, control recordings using the internal solution alone or the vehicle alone were carried out in slices from the same mice as used for test recordings.

〔Results〕

1. Hetero-LTD of excitatory synapses at layer II/III neurons of the mouse visual cortex was induced at P14-20. At P7-10 the magnitude of hetero-LTD was maximal, and became less dominant with development. Hetero-LTD was not induced at P35-41.

2. Based on the results of the paired-pulse ratio of responses and the analysis of the coefficient of variation (CV) of EPSPs, the expression locus of this form of LTD was judged as presynaptic.

3. The *N*-methyl-D-aspartate (NMDA) receptor antagonist and the Ca^{2+} chelator had no effect on hetero-LTD, but the application of the antagonist for the type 5 of metabotropic glutamate receptor (mGluR5) blocked hetero-LTD, suggesting that this form of LTD was not dependent on the activation of NMDA receptors nor the increase in postsynaptic Ca^{2+}, but dependent on the activation of mGluR5.

4. The type 1 of eCB receptors, CB_1R, was suggested to be involved in the induction of hetero-LTD, because the application of a CB_1R antagonist blocked hetero-LTD.

5. The application of brain-derived neurotrophic factor (BDNF) blocked the induction of hetero-LTD and antagonized the suppressive action of the CB_1R agonist on excitatory synaptic responses. Because BDNF is known to enhance transmitter release from presynaptic terminals, these results suggested that activated presynaptic terminals may be protected from eCB-mediated depression by the activation of presynaptic BDNF receptorsd and consequently activated synapses remain potentiated.

〔Conclusions〕

In the developing visual cortex of young mice, tetanic synaptic inputs to cortical neurons induce hetero-LTD at non-activated synapses simultaneously with homo-LTP at activated synapses. The induction of hetero-LTD is dependent on the mGluR5-endocannabinoid-CB_1R signaling pathway, and BDNF which is assumed to be released from activated presynaptic terminals may counteract the action of endocannabinoids so as not to suppress these activated terminals.

論文審査の結果の要旨

大脳皮質視覚野では求心路を高頻度で刺激すると、刺激されたシナプスの伝達効率が長期持続的に高まると同時に刺激されなかったシナプスの伝達効率が持続的に低下することが知られている。前者は同シナプス性長期増強、後者は異シナプス性長期抑圧とよばれ、視覚野の神経回路が経験によって変わる基礎過程であると想定されている。そのメカニズムについては、同シナプス性長期増強については良く知られているが、異シナプス性長期抑圧については不明であった。

本研究では、異シナプス性長期抑圧のメカニズムを明らかにするため、幼若マウス視覚野のスライス標本を用いて、皮質４層電気刺激に対する 2/3 層ニューロンのシナプス反応を電気生理学的に観測した。その結果、異シナプス性長期抑圧は生後７日から20日で誘発されるが、35-41日を過ぎると誘発されなくなること、その誘発には代謝型グルタミン酸受容体５型の活性化、及びシナプス前カンナビノイド受容体の活性化を要することが明らかとなった。さらに、刺激を受けたシナプスでは、内因性カンナビノイドの存在にもかかわらず抑制されないのは、脳由来神経栄養因子のためであることを示す結果も得た。

以上、本研究は、従来明らかでなかった異シナプス性長期抑圧のメカニズムを解明したものであり、学位の授与に値すると考えられる。