論文内容の要旨

論 文 題 目　Synaptic modulation mediated by the endocannabinoid 2-arachidonoylglycerol in the central nervous system.
(中枢神経系における内因性カンナビノイド 2–アラキドノイルグリセロールによるシナプス修飾)

氏名　谷村　あさみ

要旨

Marijuana exerts its action by binding its main active component Δ^9-tetrahydrocannabinol to cannabinoid (CB) receptors. They are seven-transmembrane-domain receptors coupled to $G_{i/o}$ protein and consist of two isoforms, CB_1 and CB_2. While the CB_1 receptor is dominantly expressed in the brain, the CB_2 receptor is expressed mainly in the immune system. Therefore, it is assumed that the CB_1 receptor is responsible for most of the psychoactivity of cannabis. *N*-arachidonylethanolamide (anandamide) and 2-arachidonylglycerol (2-AG) have been identified as major endocannabinoids, that is, the endogenous ligands for CB receptors.

A major physiological role of endocannabinoids is to mediate retrograde signaling at synapses. Endocannabinoids are released from postsynaptic neurons, travel retrogradely across the synaptic cleft, activate CB_1 receptors on presynaptic terminals, and suppress neurotransmitter release. Both short-term and long-term depression can be induced by endocannabinoids depending on the pattern and duration of CB_1 receptor activation. The endocannabinoid-mediated retrograde signaling has been found in various brain regions and is now regarded as an important and fundamental mechanism of synaptic modulation.

Endocannabinoid production and release are induced by three distinct modes of activity in postsynaptic neurons: 1) strong depolarization of postsynaptic neurons and the resultant elevation of Ca^{2+} concentration to

micromolar range, 2) strong activation of $G_{q/11}$-coupled receptors at basal intracellular Ca^{2+} level and 3) combination of postsynaptic Ca^{2+} elevation with $G_{q/11}$-coupled receptor activation. Between the two major endocannabinoids, results from pharmacological studies suggest that 2-AG rather than anandamide mediates retrograde signaling. This is based on the results that endocannabinoid-mediated retrograde suppression was prolonged by inhibitors of monoacylglycerol lipase (MGL), a major 2-AG hydrolyzing enzyme, and that the suppression was abolished by inhibitors of diacylglycerol lipase (DGL), the enzyme that synthesize 2-AG. However the inhibitors used in previous studies are not specific for MGL or DGL. Moreover, there are two isoforms of DGL, DGLα and DGLβ, and inhibitors do not discriminate the two DGL isoforms.

In the first part of my thesis work, I examined whether 2-AG mediates retrograde signaling and, if so, relative contribution of the two DGL isoforms to 2-AG synthesis. I used DGLα and DGLβ knockout mice that were generated in collaboration with Prof. Sakimura's laboratory (Niigata University). Basal amounts of 2-AG in the cerebellum, hippocampus and striatum were markedly reduced in DGLα knockout mice, whereas those were normal in DGLβ knockout mice. These results indicate that DGLα is the major 2-AG synthesizing enzyme in the brain. Then I examined whether endocannabinoid-mediated retrograde suppression was affected in the cerebellum, hippocampus and striatum of the two strains of knockout mice. I made whole-cell recordings from Purkinje cells in cerebellar slices, from pyramidal cells in hippocampal slices, from neurons in dissociated hippocampal cultures and from medium spiny neurons in striatal slices. I found that transient suppression of excitatory synaptic currents (EPSCs) or inhibitory synaptic currents (IPSCs) induced by strong depolarization of postsynaptic neurons, by strong $G_{q/11}$-coupled receptor activation, and by combined depolarization with $G_{q/11}$-coupled receptor activation were all absent in the three brain areas of DGLα knockout mice. Long-term depression was also absent in the hippocampus of DGLα knockout mice. By marked contrast, transient suppression of EPSC or IPSC induced by the three modes of endocannabinoid release was normal in the cerebellum and

hippocampus of DGLβ knockout mice. These results clearly indicate that 2-AG produced by DGLα, not by DGLβ, mediates retrograde signaling at central synapses.

In the second part of my thesis work, I examined how 2-AG is terminated after causing retrograde suppression of transmitter release. Most 2-AG is known to be hydrolyzed into arachidonic acid and glycerol by MGL that is enriched in presynaptic terminals. For this purpose, I focused on the cerebellar cortex that contains four distinct types of cannabinoid sensitive synapses. I used global MGL knockout mice and cerebellar granule cell (GC)-specific MGL knockout mice that were generated also in collaboration with Prof. Sakimura's laboratory (Niigata University). First, I examined localization of MGL in the cerebellar cortex of wild-type mice by immunofluorescence microscopic and immunogold electron microscopic analyses. MGL was expressed richly in terminals of parallel fibers (PFs), the axons of GCs, and weakly in Bergman glia, but was absent in climbing fiber (CF) terminals and GABAergic inhibitory terminals of basket cell (BC) and stellate cell (SC). Then I made whole-cell recordings from PCs in cerebellar slices and examined depolarization-induced retrograde suppression at the four types of synapses. Despite the highly selective MGL expression pattern, depolarization-induced 2-AG-mediated retrograde suppression was significantly prolonged at not only PF- PC synapse but also CF-PC synapse in GC-specific MGL knockout mice whose MGL expression in PF terminals was eliminated and MGL was present only in Bergmann glia. Moreover, 2-AG-mediated signaling at both PF-PC and CF-PC synapses was significantly shorter in GC-specific MGL-KO mice compared with global MGL-KO mice. These results indicate that MGL in PF terminals facilitates termination of 2-AG signaling not only "homosynaptically" at PFs but also "heterosynaptically" at CFs, and that MGL in Bergmann glia also contributes to termination of 2-AG signaling. To further examine the role of MGL in Bergmann glia, I prepared organotypic cerebellar slice cultures from global MGL-KO mice and introduced MGL into Bergmann glia by using a lentivirus vector. As expected, depolarization-induced retrograde suppression at PF-PC synapses was significantly shorter in slice cultures

with MGL over-expression into Bergmann glia than in control cultures with GFP expression alone. Finally, I compared depolarization-induced retrograde suppression at SC-PC synapses and that at BC-PC synapses. Retrograde suppression was significantly prolonged at SC-PC synapses that are surrounded by PFs and located close to Bergmann glial processes, but not at BC-PC synapses that are remote from MGL-rich PFs. These results indicate taht 2-AG is degraded in a synapse type-independent manner by MGL present in PFs and Bergmann glia. The results of the present study strongly suggest that MGL regulates 2-AG signaling rather broadly within a certain range of neural tissue, although MGL expression is heterogeneous and limited to a subset of nerve terminals and astrocytes.