

Forum Minireview

New Perspectives in the Studies on Endocannabinoid and Cannabis: Abnormal Behaviors Associate With CB₁ Cannabinoid Receptor and Development of Therapeutic ApplicationMichihiro Fujiwara^{1,*} and Nobuaki Egashira¹¹Department of Neuropharmacology, Faculty of Pharmaceutical Sciences, Fukuoka University, Fukuoka 814-0180, Japan

Received September 27, 2004; Accepted October 27, 2004

Abstract. Δ^9 -Tetrahydrocannabinol (Δ^9 -THC), the major psychoactive component of marijuana, induces catalepsy-like immobilization and impairment of spatial memory in rats. Δ^9 -THC also induces aggressive behavior in isolated housing stress. These abnormal behaviors could be counteracted by SR141716A, a CB₁ cannabinoid receptor antagonist. Also Δ^9 -THC inhibited release of glutamate in the dorsal hippocampus, but this inhibition could be antagonized by SR141716A in an in vivo microdialysis study. Moreover, NMDA and AMPA-type glutamate receptor enhancers improved the Δ^9 -THC-induced impairment of spatial memory. On the other hand, Δ^9 -THC markedly inhibited the neurodegeneration in experimental allergic encephalomyelitis (EAE), an animal model of multiple sclerosis and reduced the elevated glutamate level of cerebrospinal fluid induced by EAE. These therapeutic effects on EAE were reversed by SR141716A. Taken together, our results demonstrate that the inhibition of glutamate release via activation of the CB₁-cannabinoid receptor is one mechanism involved in Δ^9 -THC-induced impairment of spatial memory, and the therapeutic effect of Δ^9 -THC on EAE, and a Δ^9 -THC analog might provide an effective treatment for psychosis and neurodegenerative diseases.

Keywords: Δ^9 -tetrahydrocannabinol, CB₁ cannabinoid receptor, glutamate, spatial memory, experimental allergic encephalomyelitis

Introduction

Δ^9 -Tetrahydrocannabinol (Δ^9 -THC), the principal psychoactive component of marijuana, has been known to impair immediate memory, short-term memory, and spatial cognition in humans (1–3). Δ^9 -THC has also been reported to impair performance in the radial maze in rats (4–7). Moreover, Δ^9 -THC induces catalepsy-like immobilization and aggressive behavior (8, 9). Thus, Δ^9 -THC induces abnormal behaviors in rats. On the other hand, cannabinoids have been used to treat a variety of diseases, and recently interest has increased again. Cannabinoids have potential for the development of useful agents for the treatment of pain, emesis, asthma, multiple sclerosis (MS), and other disorders (10).

The present article introduces our recent study related

to the abnormal behaviors induced by Δ^9 -THC and the therapeutic effects of Δ^9 -THC on experimental allergic encephalomyelitis (EAE), an animal model of MS.

Involvement of the CB₁ cannabinoid receptor in Δ^9 -THC-induced abnormal behaviors in rats

Two cannabinoid receptors have been cloned: the CB₁ cannabinoid receptor in the central nervous system (11) and the CB₂ cannabinoid receptor in immune cells and peripheral tissues (12). Δ^9 -THC binds both CB₁ and CB₂ cannabinoid receptors. We previously reported that Δ^9 -THC impaired spatial memory in the eight-arm radial maze and that this impairment was reversed by the CB₁ cannabinoid receptor antagonist SR141716A (5), suggesting that the action of Δ^9 -THC is CB₁ cannabinoid receptor-mediated. We also reported that Δ^9 -THC selectively impaired working memory in a reference and working memory task using an eight-arm radial maze in which food was laid as bait in four of the eight

*Corresponding author. FAX: +81-92-863-0389
E-mail: mfuji@fukuoka-u.ac.jp

arms (5). Moreover, synthetic cannabinoid receptor agonists CP55,940 and WIN55,212-2 impaired the rats' performance in the radial maze task (13). In contrast, SR141716A alone enhanced spatial memory in the delayed task in the radial maze (14). Thus, CB₁ cannabinoid receptors appear to play an important role in learning and memory, especially working memory. We also reported that the microinjection of Δ^9 -THC impaired spatial memory when injected into the dorsal and ventral hippocampus (7). These regions have a high density of CB₁ cannabinoid receptors (15–18). Moreover, the intrahippocampal microinjection of CP55,940 impaired maze performance (13). These findings would suggest that Δ^9 -THC impairs spatial memory through direct action at CB₁ cannabinoid receptors in the dorsal and ventral hippocampus.

On the other hand, Δ^9 -THC also induces catalepsy-like immobilization and aggressive behavior in rats (8, 9). These abnormal behaviors were antagonized by SR141716A (our unpublished data). We have also found that the microinjection of Δ^9 -THC induces catalepsy-like immobilization when it is injected into the nucleus accumbens, amygdala, or hypothalamus, where the CB₁ cannabinoid receptors are expressed (unpublished data). These findings suggest that Δ^9 -THC induces abnormal behaviors through CB₁ cannabinoid receptors in the brain sites such as the nucleus accumbens, amygdala, and hypothalamus.

Involvement of the glutamatergic neuronal system in Δ^9 -THC-induced impairment of spatial memory in rats

Δ^9 -THC and WIN55,212-2 have been reported to inhibit acetylcholine (ACh) release in the dorsal hippocampus using brain microdialysis, but the inhibition effects can be antagonized by SR141716A (19, 20). Moreover, cannabinoids inhibit the release of glutamate in rat hippocampal cultures (21). Nakazi et al. (22) reported that synthetic cannabinoid receptor agonists inhibited both the electrically and Ca²⁺-induced release of serotonin (5-HT) in mouse brain cortex slices via presynaptic CB₁ cannabinoid receptors. We previously reported that Δ^9 -THC (6 mg/kg, i.p.), which impairs spatial memory, markedly reduced release of ACh and 5-HT in the dorsal or ventral hippocampus (6, 23). We also reported that cholinesterase inhibitors and 5-HT agonists improved Δ^9 -THC-induced impairment of spatial memory (6, 23). These findings suggest that the ACh and 5-HT neuronal systems may be involved in Δ^9 -THC-induced impairment of spatial memory. On the other hand, we have found that Δ^9 -THC (6 mg/kg, i.p.) inhibits the release of glutamate in the dorsal

hippocampus, and this inhibition is antagonized by SR141716A in an in vivo microdialysis study, indicating that the release of glutamate is mediated by the CB₁ cannabinoid receptor (unpublished data). Moreover, we have found that *d*-cycloserine, a partial agonist at the glycine modulatory site on the NMDA receptor, and aniracetam, a positive modulator of the AMPA receptor, improve the Δ^9 -THC-induced impairment of spatial memory (unpublished data). These findings suggest that Δ^9 -THC may impair spatial memory, at least in part, by inhibiting the release of glutamate through CB₁ cannabinoid receptors in the dorsal hippocampus.

It has been demonstrated that activation of the CB₁ cannabinoid receptor inhibits N- and P/Q-type Ca²⁺ channels in cultured hippocampal neurons (24, 25). These channels are known to be required for the release of transmissions from the hippocampal synapses (26, 27). Moreover, cannabinoids have been shown to inhibit adenylate cyclase activity (28, 29) and enhance voltage-sensitive K⁺ channels (30). These cellular effects would be expected to inhibit neurotransmitter release. Therefore it is possible that presynaptic CB₁ cannabinoid receptor activation inhibits the release of glutamate.

In addition, Δ^9 -THC and the cannabinoid receptor agonists have been shown to impair long-term potentiation (LTP) in rat hippocampal slices, a candidate mechanism for learning and memory (31–33). It has been thought that the inhibitory effect of the cannabinoids on LTP is related to the inhibition of glutamate release via presynaptic CB₁ cannabinoid receptors (34). Thus, the glutamatergic neuronal system may be involved in the Δ^9 -THC-induced impairment of spatial memory.

Involvement of the glutamatergic neuronal system in the therapeutic effect of Δ^9 -THC on EAE

EAE is an autoimmune disease of the central nervous system (CNS) that has been characterized and proposed as a valid animal model for the study of human MS (35, 36). It is widely accepted that the most common pathological abnormality in MS is an inflammatory demyelination in the CNS, but recent studies have highlighted the additional presence of axonal damage (37, 38). The cause of demyelination and axonal damage remains unclear, but several lines of evidence support the possibility that glutamate-induced excitotoxicity plays a role in these pathological changes in MS. Glutamate concentration in the cerebrospinal fluid (CSF) is reported to be elevated in patients suffering from acute MS (39, 40). In EAE, glutamate-metabolizing enzymes, glutamine synthetase, and glutamate dehydrogenase are downwardly regulated in astrocytes (41). We have found that the glutamate level of CSF is

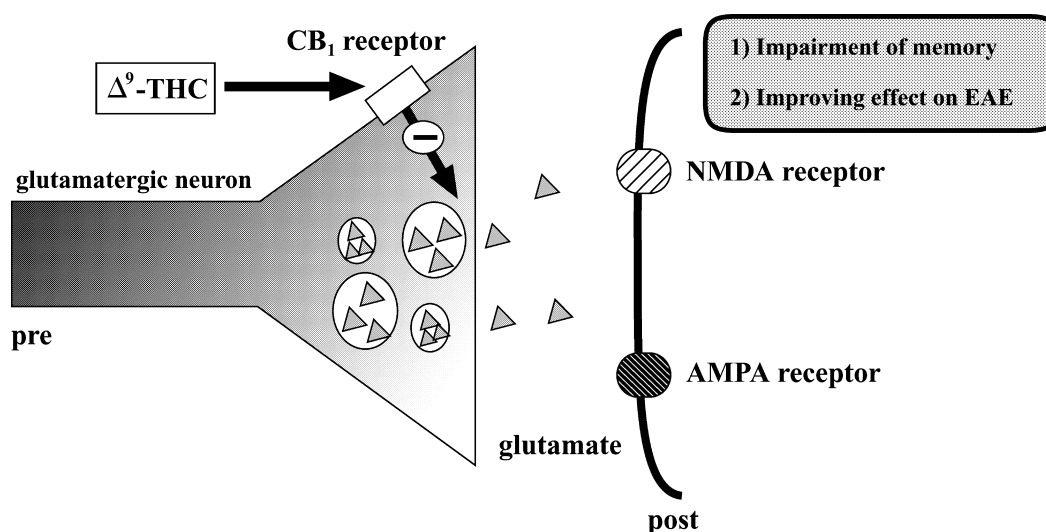


Fig. 1. Possible involvement of glutamatergic neuronal system in Δ^9 -THC-induced impairment of spatial memory and improving effect of Δ^9 -THC on EAE. The inhibition of glutamate release via activation of the CB₁ cannabinoid receptor is one mechanism in Δ^9 -THC-induced impairment of spatial memory and the therapeutic effect of Δ^9 -THC on EAE.

elevated immediately after the onset of EAE (unpublished data). Recently, amelioration of EAE by NMDA- and AMPA-type glutamate receptor antagonists was reported (42–44). Moreover, riluzole, an inhibitor of glutamate transmission, has been reported to reduce inflammation, demyelination, and axonal damage in the spinal cord and attenuate the clinical severity of EAE (45). We have also found that riluzole inhibits EAE and reduces an elevated glutamate level of CSF induced by EAE (unpublished data). These findings suggest that the glutamate neuronal system is essential to the mechanism of EAE.

Cannabinoids have been reported to inhibit both clinical and histologic EAE (46–48). We have found that Δ^9 -THC ameliorates both clinical severity of EAE and histological signs in the lumbar spinal cord and reduces the glutamate level of CSF (unpublished data). Moreover, we have found that these therapeutic effects on EAE are reversed by SR141716A, suggesting that the action of Δ^9 -THC was CB₁ cannabinoid receptor-mediated (unpublished data). Therefore, we conclude that one mechanism of the ameliorative effect of Δ^9 -THC on EAE is an inhibition of glutamate release through the CB₁ cannabinoid receptors in the CNS, in particularly the spinal cord. Thus, the glutamatergic neuronal system may be involved in the therapeutic effect of Δ^9 -THC on EAE, and Δ^9 -THC has potential use as a drug for the treatment of acute exacerbations of MS.

Conclusions

In conclusion, the present findings show that Δ^9 -THC may impair spatial memory, at least in part, by inhibiting glutamate release through the CB₁ cannabinoid receptor (Fig. 1). Also we suggested that Δ^9 -THC induces abnormal behaviors such as catalepsy-like immobilization and aggressive behavior through the CB₁ cannabinoid receptor. Moreover, we concluded that one mechanism of the improving effect of Δ^9 -THC on EAE is an inhibition of glutamate release through the CB₁ cannabinoid receptor (Fig. 1). Cannabinoid receptor agonists, and in addition antagonists/inverse agonists of this receptor, might be developed as therapeutic agents.

Acknowledgments

Part of this study was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (No. 16591174). The authors are grateful to Professor Y. Shoyama, Department of Medicinal Resources Regulation, Graduate School of Pharmaceutical Sciences, Kyushu University, for his kind supply of natural Δ^9 -tetrahydrocannabinol. The authors are grateful to Sanofi Synthelabo (Montpellier, France) for the gift of SR141716A.

References

- 1 Dornbush RL, Fink M, Freedman AM. Marijuana, memory, and perception. *Am J Psychiatry*. 1971;128:194–197.

- 2 Miller LL, Mcfarland D, Cornett TL, Brightwell D. Marijuana and memory impairment: effect on free recall and recognition memory. *Pharmacol Biochem Behav.* 1977;7:99–103.
- 3 Tinklenberg JR, Melges FT, Hollister LE, Gillespie HK. Marijuana and immediate memory. *Nature.* 1970;226:1171–1172.
- 4 Lichtman AH, Martin BR. Δ^9 -Tetrahydrocannabinol impairs spatial memory through a cannabinoid receptor mechanism. *Psychopharmacology (Berl).* 1996;126:125–131.
- 5 Mishima K, Egashira N, Hirosawa N, Fujii M, Matsumoto Y, Iwasaki K, et al. Characteristics of learning and memory impairment induced by Δ^9 -tetrahydrocannabinol in rats. *Jpn J Pharmacol.* 2001;87:297–308.
- 6 Egashira N, Mishima K, Katsurabayashi S, Yoshitake T, Matsumoto Y, Ishida J, et al. Involvement of 5-hydroxytryptamine neuronal system in Δ^9 -tetrahydrocannabinol-induced impairment of spatial memory. *Eur J Pharmacol.* 2002;445:221–229.
- 7 Egashira N, Mishima K, Iwasaki K, Fujiwara M. Intracerebral microinjections of Δ^9 -tetrahydrocannabinol: search for the impairment of spatial memory in the 8-arm radial maze in the rat. *Brain Res.* 2002;952:239–245.
- 8 Kataoka Y, Ohta H, Fujiwara M, Oishi R, Ueki S. Noradrenergic involvement in catalepsy induced by delta 9-tetrahydrocannabinol. *Neuropharmacology.* 1987;26:55–60.
- 9 Fujiwara M, Ueki S. The course of aggressive behavior induced by a single injection of delta 9-tetrahydrocannabinol and its characteristics. *Physiol Behav.* 1979;22:535–539.
- 10 Robson P. Therapeutic aspects of cannabis and cannabinoids. *Br J Psychiatry.* 2001;178:107–115.
- 11 Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature.* 1990;346:561–564.
- 12 Munro S, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. *Nature.* 1993;365:61–65.
- 13 Lichtman AH, Dimen KR, Martin BR. Systemic or intrahippocampal cannabinoid administration impairs spatial memory in rats. *Psychopharmacology (Berl).* 1995;119:282–290.
- 14 Lichtman AH. SR141716A enhances spatial memory as assessed in a radial-arm maze task in rats. *Eur J Pharmacol.* 2000;404:175–179.
- 15 Herkenham M, Lynn AB, Johnson MR, Melvin LS, de Costa BR, Rice KC. Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. *J Neurosci.* 1991;11:563–583.
- 16 Matsuda LA, Bonner TI, Lolait SJ. Localization of cannabinoid receptor mRNA in rat brain. *J Comp Neurol.* 1993;327:535–550.
- 17 Adams IB, Compton DR, Martin BR. Assessment of anandamide interaction with the cannabinoid brain receptor: SR141716A antagonism studies in mice and autoradiographic analysis of receptor binding in rat brain. *J Pharmacol Exp Ther.* 1998;284:1209–1217.
- 18 Moldrich G, Wenger T. Localization of the CB₁ cannabinoid receptor in the rat brain. An immunohistochemical study. *Peptides.* 2000;21:1735–1742.
- 19 Gessa GL, Casu MA, Carta G, Mascia MS. Cannabinoids decrease acetylcholine release in the medial-prefrontal cortex and hippocampus, reversal by SR 141716A. *Eur J Pharmacol.* 1998;355:119–124.
- 20 Nava F, Carta G, Battasi AM, Gessa GL. D₂ dopamine receptors enable Δ^9 -tetrahydrocannabinol induced memory impairment and reduction of hippocampal extracellular acetylcholine concentration. *Br J Pharmacol.* 2000;130:1201–1210.
- 21 Shen M, Piser TM, Seybold VS, Thayer SA. Cannabinoid receptor agonists inhibit glutamatergic synaptic transmission in rat hippocampal cultures. *J Neurosci.* 1996;72:169–177.
- 22 Nakazi M, Bauer U, Nickel T, Kathmann M, Schlicker E. Inhibition of serotonin release in the mouse brain via presynaptic cannabinoid CB₁ receptors. *Naunyn Schmiedebergs Arch Pharmacol.* 2000;361:19–24.
- 23 Mishima K, Egashira N, Matsumoto Y, Iwasaki K, Fujiwara M. Involvement of reduced acetylcholine release in Δ^9 -tetrahydrocannabinol-induced impairment of spatial memory in the 8-arm radial maze. *Life Sci.* 2002;72:397–407.
- 24 Twitchell W, Brown S, Mackie K. Cannabinoids inhibit N- and P/Q-type calcium channels in cultured rat hippocampal neurons. *J Neurophysiol.* 1997;78:43–50.
- 25 Shen M, Thayer SA. The cannabinoid agonist Win55,212-2 inhibits calcium channels by receptor-mediated and direct pathways in cultured rat hippocampal neurons. *Brain Res.* 1998;783:77–84.
- 26 Takahashi T, Momiyama A. Different types of calcium channels mediate central synaptic transmission. *Nature.* 1993;366:156–158.
- 27 Wheeler DB, Randall A, Tsien RW. Roles of N-type and Q-type Ca²⁺ channels in supporting hippocampal synaptic transmission. *Science.* 1994;264:107–111.
- 28 Howlett AC, Fleming RM. Cannabinoid inhibition of adenylate cyclase. Pharmacology of the response in neuroblastoma cell membranes. *Mol Pharmacol.* 1984;26:532–538.
- 29 Barg J, Fride E, Hanus L, Levy R, Matus-Leibovitch N, Heldman E, et al. Cannabinomimetic behavioural effects of and adenylate cyclase inhibition by two new endogenous anandamides. *Eur J Pharmacol.* 1995;287:145–152.
- 30 Deadwyler SA, Hampson RE, Mu J, Whyte A, Childers S. Cannabinoids modulate voltage sensitive potassium A-current in hippocampal neurons via a cAMP-dependent process. *J Pharmacol Exp Ther.* 1995;273:734–743.
- 31 Nowicky AV, Teyler TJ, Vardaris RM. The modulation of long-term potentiation by Δ^9 -tetrahydrocannabinol in the rat hippocampus, in vitro. *Brain Res Bull.* 1987;19:663–672.
- 32 Collins DR, Pertwee RG, Davies SN. Prevention by the cannabinoid antagonist, SR141716A, of cannabinoid-mediated blockade of long-term potentiation in the rat hippocampal slice. *Br J Pharmacol.* 1995;115:869–870.
- 33 Terranova JP, Michaud JC, Le Fur G, Soubrie P. Inhibition of long-term potentiation in rat hippocampal slices by anandamide and WIN55212-2: reversal by SR141716 A, a selective antagonist of CB₁ cannabinoid receptors. *Naunyn Schmiedebergs Arch Pharmacol.* 1995;352:576–579.
- 34 Schlicker E, Kathmann M. Modulation of transmitter release via presynaptic cannabinoid receptors. *Trends Pharmacol Sci.* 2001;22:565–572.
- 35 Pender MP, Sears TA. The pathophysiology of acute experimental allergic encephalomyelitis in the rabbit. *Brain.* 1984;107:699–726.
- 36 Raine CS. Biology of disease. The analysis of autoimmune demyelination: its impact on multiple sclerosis. *Lab Invest.* 1984;50:608–635.

- 37 Ferguson B, Matyszak MK, Esiri MM, Perry VH. Axonal damage in acute multiple sclerosis lesions. *Brain*. 1997;120:393–399.
- 38 Trapp BD, Peterson J, Ransohoff RM, Rudick R, Mork S, Bo L. Axonal transection in the lesions of multiple sclerosis. *N Engl J Med*. 1998;338:278–285.
- 39 Stover JF, Pleines UE, Morganti-Kossmann MC, Kossmann T, Lowitzsch K, Kempinski OS. Neurotransmitters in cerebrospinal fluid reflect pathological activity. *Eur J Clin Invest*. 1997;27:1038–1043.
- 40 Barkhatova VP, Zavalishin IA, Askarova LSh, Shavratskii VKh, Demina EG. Changes in neurotransmitters in multiple sclerosis. *Neurosci Behav Physiol*. 1998;28:341–344.
- 41 Hardin-Pouzet H, Krakowski M, Bourbonniere L, Didier-Bazes M, Tran E, Owens T. Glutamate metabolism is down-regulated in astrocytes during experimental allergic encephalomyelitis. *Glia*. 1997;20:79–85.
- 42 Bolton C, Paul C. MK-801 limits neurovascular dysfunction during experimental allergic encephalomyelitis. *J Pharmacol Exp Ther*. 1997;282:397–402.
- 43 Smith T, Groom A, Zhu B, Turski L. Autoimmune encephalomyelitis ameliorated by AMPA antagonists. *Nat Med*. 2000;6:62–66.
- 44 Pitt D, Werner P, Raine CS. Glutamate excitotoxicity in a model of multiple sclerosis. *Nat Med*. 2000;6:67–70.
- 45 Gilgun-Sherki Y, Panet H, Melamed E, Offen D. Riluzole suppresses experimental autoimmune encephalomyelitis: implications for the treatment of multiple sclerosis. *Brain Res*. 2003;989:196–204.
- 46 Lyman WD, Sonett JR, Brosnan CF, Elkin R, Bornstein MB. Delta 9-tetrahydrocannabinol: a novel treatment for experimental autoimmune encephalomyelitis. *J Neuroimmunol*. 1989;23:73–81.
- 47 Wirguin I, Mechoulam R, Breuer A, Schezen E, Weidenfeld J, Brenner T. Suppression of experimental autoimmune encephalomyelitis by cannabinoids. *Immunopharmacology*. 1994;28:209–214.
- 48 Achiron A, Miron S, Lavie V, Margalit R, Biegon A. Dexamabinol (HU-211) effect on experimental autoimmune encephalomyelitis: implications for the treatment of acute relapses of multiple sclerosis. *J Neuroimmunol*. 2000;102:26–31.