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Marijuana Receptors and Their Endogenous Ligands: from Basic Research to Diseases

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Delta-9-tetrahydrocannabinol (delta-9-THC) is a major psychoactive constituent of marijuana and is known to exert a variety of pharmacological effects on experimental animals and humans. The mechanism of action of delta-9-THC has long remained uncertain. In 1988, Devane *et al.* (1) provided evidence for the occurrence of specific binding site(s) for cannabinoids in rat brain synaptosomes using a radiolabeled synthetic cannabinoid, [^{3}H]CP55940. In 1990, Matsuda *et al.* (2) cloned a cDNA encoding a cannabinoid receptor (CB1 receptor) from a rat brain cDNA library. The CB1 receptor is present in various mammalian tissues especially in the nervous tissues and is assumed to be involved in the attenuation of neurotransmission (3). In 1993, Munro *et al.* (4) cloned a cDNA encoding another type of cannabinoid receptor (CB2 receptor) from an HL-60 cDNA library. The CB2 receptor is present mainly in the immune system and is supposed to be involved in the regulation of immune responses and/or inflammatory reactions (3), although details have not yet been elucidated. It has generally been assumed that various cannabinoids including delta-9-THC exert their biological activities mostly through acting on these cannabinoid receptors (CB1 and CB2).

The presence of specific receptor sites for cannabinoids stimulated the search for endogenous ligand(s). In 1992, Devane *et al.* (5) isolated N-arachidonoyl-ethanolamine (anandamide) from pig brain as the first endogenous cannabinoid receptor ligand. On the other hand, we isolated 2-arachidonoylglycerol (2-AG) from rat brain, and found that it acts as an endogenous cannabinoid receptor ligand (6,7). Independently and concurrently, Mechoulam *et al.* (8) also isolated 2-AG from the canine gut. It has been shown that 2-AG binds to the cannabinoid receptors (CB1 and CB2) (6-8), and exhibits several cannabimimetic activities *in vitro* and *in vivo* (8-10). Despite its possible physiological significance, however, relatively little attention has been paid to 2-AG compared with anandamide. Indeed, the physiological significance of 2-AG has very often been overlooked. Obviously, 2-AG is a noticeable molecule from a variety of

viewpoints; we have focused on 2-AG and investigated intensively the biological activities and physiological roles of 2-AG.

In 1996, we found that 2-AG induces a rapid transient increase in the intracellular free Ca^{2+} concentrations (Ca^{2+} transient) in neuroblastoma x glioma hybrid NG108-15 cells which express the CB1 receptor (11). We confirmed that the response induced by 2-AG was blocked by pretreatment of the cells with SR141716A, a cannabinoid CB1 receptor-specific antagonist, indicating that the response induced by 2-AG was mediated through the CB1 receptor (11-13). We also found that similar 2-AG-induced Ca^{2+} transients were observed in promyelocytic HL-60 cells which express the CB2 receptor. We confirmed that the response was abolished by pretreatment of the cells with SR144528, a cannabinoid CB2 receptor-specific antagonist, indicating that the response induced by 2-AG was also mediated through the CB2 receptor (14). Free arachidonic acid did not exhibit any agonistic activity in either case, indicating that arachidonic acid that may be generated from 2-AG during incubation is not involved in the response. We also confirmed that pretreatment of the cells with indomethacin, a cyclooxygenase inhibitor, or nordihydroguaiaretic acid, a lipoxygenase inhibitor, did not affect the response, suggesting that arachidonic acid metabolites are not involved.

Next, we examined the mechanism underlying the Ca^{2+} transient induced by 2-AG. We found that pretreatment of the cells with pertussis toxin abolished the response induced by 2-AG, indicating that Gi/Go is involved in the response. We also found that pretreatment of the cells with U73122, a phospholipase C inhibitor nullified the response induced by 2-AG, suggesting that phospholipase C is involved in the response induced by 2-AG. We assumed that the mechanism underlying the 2-AG-induced Ca^{2+} transient involves 2-AG binding to the cannabinoid receptors (CB1 and CB2) followed by activation of Gi/Go. The liberated beta/gamma subunit of Gi/Go then stimulates phospholipase C to enhance the production of inositol 1,4,5-trisphosphate, thereby increasing the intracellular free Ca^{2+} concentration.

The structure-activity relationship of 2-AG and other cannabinoid receptor ligands was investigated in detail using NG108-15 cells (for the CB1 receptor) and HL-60 cells (for the CB2 receptor). We found that an ether-linked analogue of 2-AG possesses substantial biological activity, indicating that the structure of 2-AG itself, but not its metabolite, is actually recognized by receptor molecules (CB1 and CB2). Noticeably, the activities of ether-linked analogues and amide bond-containing analogues of 2-AG were considerably lower than that of 2-AG, suggesting that the presence of an ester linkage is crucially important for exhibiting strong agonistic activity. In contrast to 2-AG, anandamide was found to act as a weak partial agonist toward either the CB1 receptor or the CB2

receptor. The activity of delta-9-THC, a major psychoactive constituent of marijuana, was also low. We further examined the activities of various 2-AG analogues and classical and synthetic cannabinoids. The activities of monoacylglycerols containing various saturated, monoenoic and dienoic fatty acids were very low. Among various naturally occurring analogues, 2-AG acted as the most potent agonist toward both types of receptors (CB1 and CB2). We concluded that either the CB1 receptor or the CB2 receptor is primarily a 2-AG receptor (12-14). Several pharmacological effects of delta-9-THC, a partial agonist of the cannabinoid receptors, may be attributed to the interference of the actions of the physiological ligand 2-AG.

Recently, we developed a sensitive analytical method employing HPLC to quantify monoacylglycerols (15). Using this method, we analyzed monoacylglycerols in rat brain (15). We found that a substantial amount(3.36 nmol/g tissue) of 2-AG is present in rat brain isolated after decapitation (15). However, the level of 2-AG in the brain isolated from rat killed by immersion in liquid nitrogen was 0.23 nmol/g tissue. This strongly suggests that a large amount of 2-AG was generated after decapitation. We then examined the effect of administering of central nervous system stimulants. We found that the level of 2-AG was elevated markedly in picrotoxinin-administered rat brain (4-6-fold above the control level). The generation of 2-AG may be a rather common event in stimulated brains.

Then, the mechanism underlysing rapid formation of 2-AG was examined. We found that a large amount of 2-AG was generated when brain homogenate was incubated in the presence of Ca^{2+}. Simultaneously, a large amount of arachidonic acid-containing diacylglycerol was also generated. Employing radiolabeled substrates, we obtained evidence that 2-AG was formed mainly from inositol phospholipids such as phosphatidylinositol rather than from other phospholipids such as phosphatidylethanolamine and phosphatidylcholine. We hypothesized that the main pathway for the generation of 2-AG in the brain homogenate is as follows. Inositol phospholipids were first hydrolyzed by phospholipase C to yield arachidonic acid-containing diacylglycerols. The resultant diacylglycerols were degraded by diacylglycerol lipase to yield 2-AG. Another possible route for the generation of 2-AG in the brain may be that phosphatidylinositol was first degraded by phospholipase A1 and then hydrolyzed by phospholipase C to yield 2-AG. The elucidation of the relative physiological importance and/or role allotment of these pathways in the brain awaits further investigation. In any case, whatever the detailed mechanism, it appears that the function of the cannabinoid receptors are closely related to increased inositol phospholipid metabolism in stimulated brains.

Physiological roles of 2-AG in various mammalian tissues are not yet fully

understood. We hypothesized that the physiological role of 2-AG generated through increased phospholipid metabolism such as inositol phospholipid turnover during neural excitation is as follows. 2-AG is a membrane permeable molecule; 2-AG would be released rapidly presynaptically and/or postsynaptically upon stimulation. The released 2-AG then binds to the cannabinoid CB1 receptor expressed presynaptically to attenuate the intracellular level of Ca^{2+} thereby diminishing neurotransmitter release. In support of this, we obtained evidence that 2-AG suppresses depolarization-induced elevation of the intracellular free Ca^{2+} concentration in differentiated NG108-15 cells (16). Whether this hypothesis is true should be further clarified in the future.

We also assumed that 2-AG plays an important role in the inflammatoryreactions. 2-AG, generated from inflammatory cells through enhanced inositol phospholipid turnover, binds to the cannabinoid CB2 receptor thereby eliciting subsequent biological responses such as accelerated inflammatory reactions. We obtained evidence that 2-AG activates MAP-kinase cascade in HL-60 cells. Alternatively, 2-AG may induce immunosuppression (17). Besides possible roles in the nervous system and the immune system, we also suggested that 2-AG is an important mediator in the cardiovascular system (18). When administered to experimental animals, 2-AG induces hypotension (19,20). We provided evidence that human umbilical vein endothelial cells produce and release 2-AG upon stimulation (18). 2-AG may be a novel type of vasodilator.

Several years have passed since the elucidation of 2-AG as an endogenous cannabinoid receptor ligand. However, information concerning 2-AG is still limited as compared with anandamide. Cannabinoid receptors are physiologically essential receptor molecules. It has been shown that the cannabinoid CB1 receptor is very often lost in the striatum of the patient suffering from Huntington's disease. Details of the physiological roles of 2-AG, the endogenous natural ligand for the cannabinoid receptors, in the nervous system, immune system as well as the cardiovascular system should be clarified in the near future.

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