Evidence That the Cannabinoid CB1 Receptor Is a 2-Arachidonoylglycerol Receptor

STRUCTURE-ACTIVITY RELATIONSHIP OF 2-ARACHIDONOYLGLYCEROL, ETHER-LINKED ANALOGUES, AND RELATED COMPOUNDS*

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An endogenous cannabimimetic molecule, 2-arachidonoylglycerol, induces a rapid, transient increase in intracellular free Ca$^{2+}$ concentrations in NG108–15 cells through a cannabinoid CB1 receptor-dependent mechanism. We examined the activities of 24 relevant compounds (2-arachidonoylglycerol, its structural analogues, and several synthetic cannabinoids). We found that 2-arachidonoylglycerol is the most potent compound examined so far: its activity was detectable from as low as 0.3 nM, and the maximal response induced by 2-arachidonoylglycerol exceeded the responses induced by others. Activities of HU-210 and CP55940, potent cannabinoid receptor agonists, were also detectable from as low as 0.3 nM, whereas the maximal responses induced by these compounds were low compared with 2-arachidonoylglycerol. Anandamide was also found to act as a partial agonist in this assay system. We confirmed that free arachidonic acid failed to elicit a response. Furthermore, we found that a metabolically stable ether-linked analogue of 2-arachidonoylglycerol possesses appreciable agonistic activity, although its activity was apparently lower than that of 2-arachidonoylglycerol. We also confirmed that pretreating cells with various cannabinoid receptor agonists nullified the response induced by 2-arachidonoylglycerol, whereas pretreating cells with other neurotransmitters or neuromodulators did not affect the response. These results strongly suggested that the cannabinoid CB1 receptor is originally a 2-arachidonoylglycerol receptor, and 2-arachidonoylglycerol is the intrinsic physiological ligand for the cannabinoid CB1 receptor.

It is well known that Δ$^9$-tetrahydrocannabinol (Δ$^9$-THC), a psychoactive ingredient of marijuana, possesses a variety of pharmacological activities in vitro and in vivo (1), although, until recently, the mechanism of the action of Δ$^9$-THC had long been unelucidated. In 1988, Devane et al. (2) provided evidence that a specific binding site(s) for cannabinoids is present in the brain. Soon after, Matsuda et al. (3) cloned a cDNA encoding a cannabinoid receptor (CB1) from a rat brain cDNA library. These findings raised the possibility that at least part of the action of Δ$^9$-THC is mediated through such a specific receptor and prompted the search for endogenous cannabinoid receptor ligands in mammalian tissues.

In 1992, Devane et al. (4) isolated N-arachidonylethanolamine (anandamide) from porcine brain as the first endogenous cannabinoid receptor ligand. They demonstrated that anandamide exhibits several cannabimimetic activities in vitro and in vivo (4, 5). So far, a number of studies have been carried out on anandamide, and it has been assumed that anandamide is one of the important lipid mediators in the nervous system as well as in other systems (5). However, we (6, 7) and others (8–11) have found that the levels of anandamide are very low in several mammalian tissues. In addition, the biosynthetic pathways for anandamide, either the N-acylphosphatidylethanolamine pathway (6, 7, 11–14) or the condensation pathway (7, 15–18), do not appear able to provide large amounts of anandamide, at least under normal conditions in living tissues, because the availability of the substrates are very low. Furthermore, several investigators demonstrated that anandamide is produced mainly in the post-mortem period in the brain (8, 10). Thus, the physiological significance or meaning of anandamide, especially in the brain, has been questioned recently despite its high binding affinity toward the cannabinoid receptor(s).

On the other hand, several years ago, we found that 2-arachidonoylglycerol, an arachidonic acid-containing monoacylglycerol, possesses binding affinity toward the cannabinoid receptor in rat brain synaptosomes and that a rat brain contains a significant amount of arachidonoylglycerol (19, 20). Indeed, the level of arachidonoylglycerol in the brain was found to be 800 times higher than that of anandamide present in the same tissue (7, 20). We suggested the possibility that 2-arachidonoylglycerol is an endogenous ligand for the cannabinoid receptor in the brain (19, 20). Mechoulam et al. (21) also isolated 2-arachidonoylglycerol from canine gut as another candidate for an endogenous cannabinoid receptor ligand; they demonstrated that 2-arachidonoylglycerol possesses binding activity toward cannabinoid receptors expressed on COS-7 cells transfected with cannabinoid receptor genes and induces the inhibition of adenylate cyclase in mouse spleen cells and twitch response in mouse vas deferens. Moreover, in recent studies, we found that 2-arachidonoylglycerol induces a rapid, transient elevation of intracellular free Ca$^{2+}$ concentration ([Ca$^{2+}$]) through a cannabinoid CB1 receptor-dependent mechanism and proposed that the cannabinoid CB1 receptor is originally a 2-arachidonoylglycerol receptor (22, 23). Thus, the physiologi-
cal significance of 2-arachidonoyl glycerol came to receive increased attention (19–33). Despite its possible physiological importance, however, the available information concerning this novel type of bioactive lipid is still limited. In addition, it is not clear whether or not 2-arachidonoyl glycerol itself, but not its metabolites, is actually implicated in the observed biological effects, especially in cases of prolonged incubation of cells or in vivo experiments. In some cases, apparently, it is not easy to interpret the obtained experimental results because of the susceptibility of 2-arachidonoyl glycerol to hydrolyzing enzymes, such as monoacylglycerol lipase.

In this study, we examined the activities of 2-arachidonoyl glycerol and its ether-linked metabolically stable analogues, as well as other structurally related compounds, to stimulate NG108–15 cells. We found that an ether-linked analogue of 2-arachidonoyl glycerol, but not that of 1(3)-arachidonoylglycerol, exhibits appreciable cannabimimetic activity, although its activity was weak compared with that of 2-arachidonoyl glycerol. The results obtained here provided strong evidence that the structure of 2-arachidonoyl glycerol is strictly recognized by the cannabinoid CB1 receptor.

EXPERIMENTAL PROCEDURES

**Chemicals**—Arachidonic acid, palmitic acid, oleic acid, linoleic acid, γ-linolenic acid, eicosatrienoic acid (n-3), docosatetraenoic acid (n-6), docosahexaenoic acid (n-3), L-glutamic acid, acetyl chloride, taurine, lysophosphatidic acid (1-oleyl), prostaglandin E2, and essentially fatty acid-free bovine serum albumin were purchased from Sigma. 1,3-Propanediol, hydroxyacetone, Fura-2/AM, benzaldehyde, glycine, adenosine, adenosine-5’-triphosphate (ATP) disodium salt, dopamine hydrochloride, (R)-(-) norepinephrine hydrochloride, (R)-(-) epinephrine, serotonin creatinine sulfate, histamine dihydrochloride, and butylated hydroxytoluene (BHT) were supplied from Wako Pure Chem. Ind. (Osaka, Japan). Eicosatrienoic acid (n-9), eicosatetraenoic acid, and (R)-1-methanandamide were from Cayman Chemical Co. (Ann Arbor, MI). SR141716A was acquired from Biomol (Plymouth Meeting, PA). CP55940 and HU-210 were purchased from Toecis (Bristol, United Kingdom). (S)(+)-2-Amino-1-propanol, (S)(+)-2,2-dimethyl-1,3-dioxolane-4-methanol (1,2-O-isopropylidene-sn-glycerol), (R)(-) 2,2-dimethyl-1,3-dioxolane-4-methanol (2,3-O-isopropylidene-sn-glycerol), and (R)(-) 1,2-dimethyl-1,3-dioxolane-4-methanol (2,3-O-isopropylidene-sn-glycerol) were purchased from Tokyo Kasei Kogyo Co. (Tokyo, Japan). WIN55212–2 and γ-aminobutyric acid (GABA) were from Research Biochemicals (Natick, MA). Platelet-activating factor (1-hexadecyl) and bradykinin were obtained from Peptide Institute (Osaka, Japan). Rabbit factor IX was obtained from the Toyo Jozo Co., Ltd. (Tokyo, Japan).

Synthesis of Propanediol-type Analogues of 1(3)-Arachidonoylglycerol, Anandamide, and (S)-1-Methanandamide—3-Hydroxypropyl arachidonate (a 1,3-propanediol-type analogue of 1(3)-arachidonoylglycerol) was prepared from 1,3-propanediol and arachidonic anhydride using dimethylaminopyridine as a catalyst and purified by TLC using petroleum ether:diethyl ether:acetic acid (20:80:1, v/v) as the solvent system (Rf, 0.58). In order to prepare 2-hydroxypropyl arachidonate (a 1,2-propanediol-type analogue of 1(3)-arachidonoylglycerol), 2-oxopropyl arachidonate was prepared from hydroxyacetone and arachidonic anhydride and purified by TLC using petroleum ether:diethyl ether:acetic acid (65:35:1, v/v) as the solvent system (Rf, 0.41). The resultant 2-oxopropyl arachidonate was treated with NaH in dry diethyl ether to yield 2-hydroxypropyl arachidonate, which was purified by TLC using petroleum ether:diethyl ether:acetic acid (65:35:1, v/v) as the solvent system (Rf, 0.20). The structure of 2-hydroxypropyl arachidonate was confirmed by NMR and electron impact mass spectrometry. Anandamide was synthesized from arachidonic anhydride and ethanolamine and purified by TLC as described previously (7). (S)-1-Methanandamide was synthesized from arachidonic anhydride and (S)-1-(2-amino-2-oxopropyl) arachidonate and purified by TLC using petroleum ether:diethyl ether:acetic acid (30:40:20:1, v/v) as the solvent system (Rf, 0.56).

Synthesis of Ether-linked Analogues of 2-Arachidonoylglycerol and 1(3)-Arachidonoylglycerol—Arachidonic acid was dissolved in chilled, dehydrated diethyl ether and treated with LiAlH4 while stirring for 1 h under an atmosphere of argon to yield eicosatetraenyl iodide. After adding ethyl acetate to remove excess LiAlH4, the mixture was filtered through Celite and then washed, first with distilled water and next with saturated Na2SO4 aqueous solution. The organic layer was dried over MgSO4 and evaporated to dryness. The residue was dissolved in dichloromethane and purified by silica gel column chromatography using hexane:ethyl acetate (5:1, v/v) as the elution solvent. Purified eicosatetraenyl iodide was then dissolved in dehydrated N,N-dimethylaniline and treated with methyltriphenylphosphonium iodide for 20 min to yield eicosatetraenyl iodide. Eicosatetraenyl iodide was purified by silica gel column chromatography (hexane:ethyl acetate, 20:2, v/v). 2-Eicosatetraenyl-1,3-benzylideneglycerol (an ether-linked analogue of 2-arachidonoyl-1,3-benzylideneglycerol) was obtained by condensing eicosatetraenyl iodide and 1,3-benzylideneglycerol using dimethylformamide as the solvent and Ag2O and tetraethylammonium iodide as catalysts. After stirring at 90 °C for 6 h under argon, the mixture was diluted with ethyl acetate and distilled water. The silver generated was removed by filtering through Celite, and the filtrate was washed, first with distilled water and then with saturated Na2SO4 aqueous solution. The organic layer was dried over MgSO4 and evaporated to dryness. The residue was dissolved in dichloromethane and purified by silica gel column chromatography using hexane:ethyl acetate (5:1, v/v) as the elution solvent. 2-Eicosatetraenylglycerol (an ether-linked analogue of 2-arachidonoylglycerol) was prepared from 2-eicosatetraenyl-1,3-benzylideneglycerol by treatment with boric acid and boric acid trimethyl ester, as in the preparation of 2-arachidonoylglycerol. 2-Eicosatetraenylglycerol was purified by silica gel column chromatography and then by TLC using ethyl acetate as the solvent (Rf, 0.48). To prepare 13-eicosatetraenylglycerol, rac-1,2-O-isopropylidene-glycerol was employed in the synthesis of 2-eicosatetraenylglycerol, and the structures of 2-eicosatetraenylglycerol and 13-ethoxyeicosatrienylglycerol were confirmed by NMR and electron impact mass spectrometry.

Synthesis of a Methylene-linked Analogue of 2-Arachidonoylglycerol—A methylene-linked analogue of 2-arachidonoylglycerol (2-hydroxymethyl-(all Z)-7,10,13,16-docosatetraen-1-ol) was prepared as follows: ethyl arachidonate was reduced with LiAlH4 in dry diethyl ether under normal conditions in the presence of butylated hydroxytoluene as an antioxidant and in a nitrogen atmosphere. The product eicosatetraenyl iodide, which was isolated by silica gel column chromatography using hexane:ethyl acetate (98:2, v/v) as the elution solvent and converted to a diethyl malonate derivative as follows: to a suspension of oil-free sodium hydride in dry tetrahydrofuran and dry N,N-dimethylformamide, a solution of dry diethyl acetylenedicarboxylate in dry diethyl ether and 2-hydroxypropyl arachidone was treated with NaBH4 to yield 2-hydroxypropyl arachidone, was then purified by TLC using petroleum ether:diethyl ether:acetic acid (20:80:1, v/v) as the solvent system (Rf, 0.58). In order to prepare 2-hydroxypropyl arachidonate (a 1,2-propanediol-type analogue of 1(3)-arachidonoylglycerol), 2-oxopropyl arachidonate was prepared from hydroxyacetone and arachidonic anhydride and purified by TLC using petroleum ether:diethyl ether:acetic acid (65:35:1, v/v) as the solvent system (Rf, 0.41). The resultant 2-oxopropyl arachidonate was treated with NaH in dry diethyl ether to yield 2-hydroxypropyl arachidonate, which was purified by TLC using petroleum ether:diethyl ether:acetic acid (65:35:1, v/v) as the solvent system (Rf, 0.20). The structure of 2-hydroxypropyl arachidonate was confirmed by NMR and electron impact mass spectrometry. NG108–15 cells were kindly donated by Prof. H. Higashida (Kanazawa University School of Medicine, Kanazawa, Japan). NG108–15 cells were grown at 37 °C in Dulbecco’s modified Eagle’s
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We then examined the activities of propanediol-type analogues of arachidonoylglycerols. We found that 2-hydroxypro pyl arachidonate, one of two hydroxy groups lacking analogues of 1(3)-arachidonoylglycerol, possesses substantial agonistic activity (Fig. 2H); its activity was almost comparable to that of 1(3)-arachidonoylglycerol. Interestingly, the activity of 3-hydroxypropyl arachidonate (Fig. 2I), another analogue of 1(3)-arachidonoylglycerol, was found to be much weaker than that of 2-hydroxypropyl arachidonate, suggesting that the presence of a free hydroxy group adjacent to the ester linkage is essential in exhibiting strong agonistic activity.

Next, we examined the activities of anandamide and its analogues. As shown in Fig. 2J, we confirmed that anandamide induces the elevation of \([\text{Ca}^{2+}]_i\). The response was detectable from as low as 3 nM, whereas the magnitude of the response induced by anandamide was not as pronounced as that induced by 2-arachidonoylglycerol even at high concentrations. We also found that (R)-1-methanandamide (Fig. 2K) exhibits agonistic activity comparable to that of anandamide, although the activity of (S)-1-methanandamide (Fig. 2L) appears to be somewhat lower than those of anandamide and (R)-1-methanandamide.

The activities of 2-monoacylglycerols containing various species of saturated and unsaturated fatty acids were next examined. As shown in Fig. 2M, 2-eicosatetraynoylglycerol, an eicosatetraenoic acid-containing analogue of 2-arachidonoylglycerol, failed to exhibit appreciable agonistic activity even at high concentrations, indicating that the presence of double bonds, but not triple bonds, is indispensable for agonistic activity. We also found that saturated or monoenoic 2-monoacylglycerols, such as 2-palmitoylglycerol (data not shown) and 2-deoxyglycerol (Fig. 2N), were inactive. The activities of 2-linoleoylglycerol (Fig. 2O) and 2-γ-linolenoylglycerol (Fig. 2P) were also very low, suggesting that the acyl moiety of 2-arachidonoylglycerol is strictly recognized by the receptor.

One striking observation shown here is the marked difference in the activities of three species of 2-eicosatrienoylglycerols. As shown in Fig. 2Q, the activity of 2-(11,14,17-eicosa trienoyl(n-3))glycerol was very low. On the other hand, the activity of 2-(8,11,14-eicosa trienoyl(n-6))glycerol (Fig. 2R) was greater than that of 2-(11,14,17-eicosatrienoyl(n-3))glycerol, although its activity was still weaker than that of 2-arachidonoylglycerol. Surprisingly, 2-(5,8,11-eicosatrienoyl(n-9))glycerol possessed strong agonistic activity, which was almost comparable to that of 2-arachidonoylglycerol (2-(5,8,11,14-eicosatetraenoyl(n-6))glycerol) (Fig. 2S), suggesting that the presence of the double bond at the Δ5 position of the C20 fatty chain is essential. In keeping with this, we found that 2-(5,8,11,14,17-eicosapentaenoyl(n-3))glycerol exhibits substantial agonistic activity (Fig. 2T). In contrast to these C20 polyunsaturated fatty acid-containing 2-monoacylglycerols, C22 polyunsaturated fatty acid-containing 2-monoacylglycerols, such as 2-docosatetraenoyl(n-6)glycerol (Fig. 2U) and 2-docosahexaenoyl(n-3)glycerol (Fig. 2V), failed to exhibit strong agonistic activities.

We then examined the activities of several synthetic cannabino ids and compared their activities to that of 2-arachidonoylglycerol. We found that HU-210 and CP55940 exhibited appreciable agonistic activities (Fig. 2, W and X). The response was detectable from as low as 0.3 nM, with a plateau at around 100 nM in each case. However, the maximal responses induced by these cannabinoids are apparently smaller than that induced by 2-arachidonoylglycerol.

To confirm that the response induced by 2-arachidonoylglycerol is mediated through the cannabinoid CB1 receptor, we examined the effects of cell pretreatment with SR141716A, a cannabinoid CB1 receptor-specific antagonist (or an inverse agonist). As shown in Fig. 3, the response induced by 1 μM...
2-arachidonoylglycerol or 10 μM 2-eicosatetraenoylglycerol, an ether analogue of 2-arachidonoylglycerol, was inhibited by cell pretreatment with 1 μM SR141716A. We also confirmed that SR141716A (1 μM) blocked the response induced by either HU-210 (10 μM) or CP55940 (10 μM) (data not shown).

Finally, we examined the effects of cell pretreatment with...
FIG. 2. The activities of 2-arachidonoylglycerol, its structural analogues, and several synthetic cannabinoids to induce rapid, transient increases in \([\text{Ca}^{2+}]_i\) in NG108–15 cells. Cells, loaded with Fura-2/AM, were stimulated with 2-arachidonoylglycerol or other compounds in the presence of CaCl_2 (1 mM), and changes in \([\text{Ca}^{2+}]_i\) were analyzed in CAF-100. The mean and S.D. were calculated from the results of four separate experiments.
various kinds of neurotransmitters or neumodulators and related compounds (final concentration, 10 m\(\mu\)) known to interact with specific receptors on the response induced by 10 m\(\mu\) 2-arachidonoylglycerol. As summarized in Table I, the cell pretreatment with various cannabinoid receptor agonists, such as \(\Delta^9\)-THC, HU-210, WIN55212–2, and CP55940, completely blocked the response induced by 2-arachidonoylglycerol, probably through the desensitization of the receptor molecule. On the other hand, cell pretreatment with various compounds other than cannabinoid receptor agonists did not affect the response induced by 2-arachidonoylglycerol.

DISCUSSION

In preceding studies, we investigated the mechanism and physiological meaning of 2-arachidonoylglycerol-induced transient increases in [Ca\(^{2+}\)] in NG108–15 cells. We established that the response induced by adding 2-arachidonoylglycerol to the cells is mediated through the cannabinoid CB1 receptor and G\(_i\) or G\(_\alpha\) (22, 23), yet the detailed mechanism is not yet fully understood. One important issue to be verified is whether or not 2-arachidonoylglycerol itself, but not its metabolites, is actually involved in this rapid cellular response. Here, we provided clear evidence to show that this is the case. First, a metabolically stable analogue of 2-arachidonoylglycerol, 2-eicosatetraenoylglycerol, was found to exhibit appreciable agonistic activity (Fig. 2E). Second, free arachidonic acid is not capable of inducing the response (Fig. 2D). In addition, we already confirmed that cell pretreatment with either indomethacin or nordihydroguaiaretic acid does not affect the cellular response (22). Thus, it is evident that the structure of 2-arachidonoylglycerol itself is recognized by the cannabinoid CB1 receptor as a receptor agonist.

Importantly, among various cannabimimetic molecules, 2-arachidonoylglycerol exhibited the highest agonistic activity. We detected its activity from as low as 0.3 nM, and the magnitude of the response induced by 2-arachidonoylglycerol was the most prominent, compared with the responses of the other compounds (Fig. 2). HU-210 and CP55940 also exhibited appreciable agonistic activities from as low as 0.3 nM (Fig. 2, W and X), whereas these compounds were shown to act as partial agonists similar to \(\Delta^9\)-THC (23) and anandamide (Fig. 2F). WIN55212–2 has also been shown to exhibit strong agonistic activity, whereas its activity was detectable only above 10 nM (22). These findings that the various cannabimimetic molecules are less active, compared with 2-arachidonoylglycerol, strongly support that the cannabinoid CB1 receptor is originally a 2-arachidonoylglycerol receptor and that 2-arachidonoylglycerol is the intrinsic physiological ligand.

Table II summarizes the preferred structures of monoacylglycerols and their structural analogues as cannabinoid receptor agonists. Glycerol is the most suitable head group, and the 2-isomer is preferable over the 1(3)-isomer. As for the fatty acyl constituent of monoacylglycerols, among those examined, although the activity of eicosatrienoic acid (n-9)-containing species was almost comparable to that of arachidonic acid-containing species. Because the activities of 2-eicosatrienoyl(n-6)glycerol, 2-eicosatrienoyl(n-3)glycerol and 2-docosatetraenoyl(n-6)glycerol are apparently lower than those of 2-eicosatrienoyl(n-9)glycerol and 2-eicosapentaenoyl(n-3)glycerol (Fig. 2), we assume that the structure near the ester linkage, such as the presence of the double bond at the \(\Delta^5\) position rather than the structure near the methyl end, is crucially important, probably for some characteristic conformation of the agonistic molecules.

Another important feature of the structural requirement is that the presence of an ester linkage, especially one that is adjacent to a free hydroxy group, is essential for strong agonistic activity. A methylene-linked analogue of 2-arachidonoylglycerol is actually involved in this rapid cellular response. Here, whether or not 2-arachidonoylglycerol itself, but not its metabolites, is actually involved in this rapid cellular response.

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glycerol possesses only weak agonistic activities (Fig. 2G). Furthermore, the activity of an ether-linked analogue of 2-arachidonoylglycerol was also considerably lower than that of 2-arachidonoylglycerol (Fig. 2E). The activity of 3-hydroxypropyl arachidonate, which lacks an adjacent free hydroxy group, was considerably lower than that of an adjacent free hydroxy group-containing analogue, 2-hydroxypropyl arachidonate (Fig. 2, H and I). It is possible, therefore, that either the oxygen atoms in the ester bond or the adjacent free hydroxy group may have close interaction with the receptor molecule. Another possibility may be that the carbonyl group of the ester bond and an adjacent free hydroxy group of the glycerol moiety are linked by a hydrogen bond under some conditions; this possibility is predicted by computer analysis evaluating the minimum energy conformation of a single molecule of 2-arachidonoylglycerol in a vacuum: one of the most stable conformations of 2000 conformations has an intramolecular hydrogen bond (O–H = 2.1 Å). Such a linkage, possible in the ester-linked compound but not in ether-linked analogues and methylene-linked analogues, is effective in fixing the glycerol-head group and yields a ring structure consisting of three carbon atoms, three oxygen atoms, and a hydrogen atom. It will be of value to examine whether or not such a ring structure can be formed in 2-arachidonoylglycerol molecules under physiological conditions, especially at the receptor site, and whether or not such a possible ring structure is implicated in the induction of strong agonistic activities.

Since the discovery of the cannabinoid receptor gene and an endogenous cannabinoid receptor ligand, anandamide, numerous studies have been conducted on cannabinoid receptors and their endogenous ligands. It is becoming evident that the cannabinoid receptor-endogenous ligand system plays important physiological roles in the nervous system. Herkenham (35) reported that whole brain cannabinoid receptor density is similar to the whole brain densities of receptors for amino acid transmitters, such as glutamic acid and GABA; this observation leads us to postulate that the endogenous cannabinoid receptor ligand(s) is also a common molecule and abundantly present in the brain, like glutamic acid and GABA. 2-Arachidonoylglycerol, but not anandamide, meets this requirement. In previous studies, we (19, 20) and others (28) found that the level of 2-arachidonoylglycerol in the brain is on the order of nmol/g of tissue, which is several hundred times higher than that of anandamide in the same tissue. Indeed, 2-arachidonoylglycerol is one of the major species of monoacylglycerols in the brain and other tissues (31). Furthermore, we recently found that 2-arachidonoylglycerol suppresses the activation of differentiated NG108–15 cells upon depolarization (29). Furthermore, Stella et al. (28) reported that 2-arachidonoylglycerol reduces long term potentiation in hippocampal slices. In vivo administration of 2-arachidonoylglycerol has been shown to induce analgesia, immobility, and reduction of spontaneous activity in mice (21). Thus, there is increasing evidence that 2-arachidonoylglycerol plays important physiological roles in the attenuation of neurotransmission and the protection of neuronal cells presumably with the cooperation of other inhibitory neurotransmitters or neuromodulators, such as GABA and adenosine.

In conclusion, we obtained clear evidence that the cannabinoid CB1 receptor is originally a 2-arachidonoylglycerol receptor and that 2-arachidonoylglycerol is the intrinsic physiological ligand for the cannabinoid CB1 receptor. 2-Arachidonoylglycerol is assumed to be derived principally from ATP. In any case, the cannabinoid CB1/2-arachidonoylglycerol receptor-dependent negative feedback control system appears to be of great physiological significance, because sustained activation of neuronal cells is known to cause cell exhaustion and may lead to neuronal cell death. In relation to this, we recently found that 2-arachidonoylglycerol suppresses the activation of differentiated NG108–15 cells upon depolarization (29). Furthermore, Stella et al. (28) reported that 2-arachidonoylglycerol reduces long term potentiation in hippocampal slices. In vivo administration of 2-arachidonoylglycerol has been shown to induce analgesia, immobility, and reduction of spontaneous activity in mice (21). Thus, there is increasing evidence that 2-arachidonoylglycerol plays important physiological roles in the attenuation of neurotransmission and the protection of neuronal cells presumably with the cooperation of other inhibitory neurotransmitters or neuromodulators, such as GABA and adenosine.

TABLE II
Preferred structures of monoacylglycerols and their structural analogues as cannabinoid receptor agonists

| Backbone | Glycerol > ethyleneglycol, propanediol |
| Position | 2-Isomer > 1-isomer, 3-isomer |
| Bond     | Ester > ether > methylene, amide |
| Acyl moiety | 20:4(n-6) > 20:3(n-9) > 20:5(n-3) > 20:3(n-6) > 20:3(n-3), 22:4(n-6), 22:6(n-3), 18:1(n-9), 18:1(n-9), 16:0 |

* From the data in Ref. 23.

The most striking and noticeable issue concerning 2-arachidonoylglycerol is that this unique lipid molecule links enhanced inositol phospholipid metabolism in stimulated neuronal cells with the function of cannabinoid receptors expressed mainly on presynaptic membranes (35, 41, 42). As described previously, 2-arachidonoylglycerol is formed in neuronal cells upon stimulation (25, 28) and in a brain homogenate upon the addition of Ca^{2+} (31), and the cannabinoid CB1 receptor is known to participate in the attenuation of neurotransmission (41, 42). Such a linkage should be effective in calming some neurons after excitation, which leads to negative feedback control of neurotransmission in some synapses in which the cannabinoid CB1/2-arachidonoylglycerol receptor is present. Similar negative feedback control mechanism may operate in the case of adenosine, which is assumed to be derived principally from ATP. In any case, the cannabinoid CB1/2-arachidonoylglycerol receptor-dependent negative feedback control system appears to be of great physiological significance, because sustained activation of neuronal cells is known to cause cell exhaustion and may lead to neuronal cell death. In relation to this, we recently found that 2-arachidonoylglycerol suppresses the activation of differentiated NG108–15 cells upon depolarization (29). Furthermore, Stella et al. (28) reported that 2-arachidonoylglycerol reduces long term potentiation in hippocampal slices. In vivo administration of 2-arachidonoylglycerol has been shown to induce analgesia, immobility, and reduction of spontaneous activity in mice (21). Thus, there is increasing evidence that 2-arachidonoylglycerol plays important physiological roles in the attenuation of neurotransmission and the protection of neuronal cells presumably with the cooperation of other inhibitory neurotransmitters or neuromodulators, such as GABA and adenosine.

In conclusion, we obtained clear evidence that the cannabinoid CB1 receptor is originally a 2-arachidonoylglycerol receptor and that 2-arachidonoylglycerol is the intrinsic physiological ligand for the cannabinoid CB1 receptor. 2-Arachidonoylglycerol is assumed to play important modulatory roles in the attenuation of neurotransmission and the protection of neuronal cells presumably with the cooperation of other inhibitory neurotransmitters or neuromodulators, such as GABA and adenosine.

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