New Perspectives in the Studies on Endocannabinoid and Cannabis: 2-Arachidonoylglycerol as a Possible Novel Mediator of Inflammation

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Abstract. 2-Arachidonoylglycerol is an endogenous ligand for the cannabinoid receptors. To date, two types of cannabinoid receptors (CB₁ and CB₂) have been identified. The CB₁ receptor is assumed to be involved in the attenuation of synaptic transmission. On the other hand, the physiological roles of the CB₂ receptor, which is abundantly expressed in several types of inflammatory cells and immunocompetent cells, have not yet been fully elucidated. Recently, we investigated in detail possible physiological roles of the CB₂ receptor and 2-arachidonoylglycerol in inflammation. We found that 2-arachidonoylglycerol induces the activation of p42/44 and p38 mitogen-activated protein kinases and c-Jun N-terminal kinase; actin rearrangement and morphological changes; augmented production of chemokines in HL-60 cells; and the migration of HL-60 cells differentiated into macrophage-like cells, human monocytes, natural killer cells, and eosinophils. We also found that the level of 2-arachidonoylglycerol in mouse ear is markedly elevated following treatment with 12-O-tetradecanoylphorbol 13-acetate, which induces acute inflammation. Notably, the inflammation induced by 12-O-tetradecanoylphorbol 13-acetate was blocked by treatment with SR144528, a CB₂-receptor antagonist. Similar results were obtained with an allergic inflammation model in mice. These results strongly suggest that 2-arachidonoylglycerol plays essential roles in the stimulation of various inflammatory reactions in vivo.

Keywords: cannabinoid, Δ⁹-tetrahydrocannabinol, 2-arachidonoylglycerol, anandamide, inflammation

Introduction

Δ⁹-Tetrahydrocannabinol (Δ⁹-THC) (Fig. 1), a major psychoactive ingredient of marijuana, has been shown to exhibit a variety of pharmacological activities in vitro and in vivo (1). For instance, the administration of Δ⁹-THC to experimental animals induces reduced spontaneous motor activity, immobility, analgesia, and impairment of short-term memory. Δ⁹-THC also exerts profound effects on several biological systems other than the nervous system, such as the immune system; for example, Δ⁹-THC induces the suppression of immune responses in experimental animals. The mechanisms underlying these actions of Δ⁹-THC remained ambiguous until the late 1980s.

In 1988, Devane et al. (2) provided clear evidence that a specific binding site for cannabinoids is present in rat brain synaptosomes using [³H]CP55940 (Fig. 1), a radiolabeled synthetic cannabinoid, as a ligand. It thus became apparent that Δ⁹-THC binds to a specific binding site(s), if not all, thereby eliciting a variety of pharmacological responses. In 1990, Matsuda et al. (3) reported the cloning of a cDNA encoding a cannabinoid receptor (CB₁) from a rat brain cDNA library. Soon after, Munro et al. (4) cloned another cDNA encoding a cannabinoid receptor (CB₂) from a promyelocytic leukemia HL-60 cell cDNA library. The CB₁ and CB₂ receptors share 44% overall identity (68% identity for the transmembrane domains), and both belong to the seven-transmembrane, G protein-coupled receptor super family.

The CB₁ receptor is abundantly expressed in the nervous system including the brain. Among brain regions, the CB₁ receptor is particularly abundant in the substantia nigra, globus pallidus, molecular layer of the cerebellum, hippocampus, and cerebral cortex; evidence
is accumulating that the CB\textsubscript{1} receptor is involved in the regulation of synaptic transmission (5). On the other hand, the CB\textsubscript{2} receptor is predominantly expressed in lymphoid tissues such as the spleen, tonsils, and lymph nodes and several types of leukocytes. The CB\textsubscript{2} receptor is assumed to participate in the regulation of inflammatory reactions and immune responses, although the details still remain rather obscure. The elucidation of the exact physiological functions of the CB\textsubscript{2} receptor are, therefore, essential for a better understanding of the precise regulatory mechanism of inflammatory reactions and immune responses. In this review, we focused on possible physiological roles of the CB\textsubscript{2} receptor and its endogenous ligand, especially 2-arachidonoylglycerol (2-AG), in inflammatory reactions.

**Endogenous cannabinoid receptor ligands**

To date, two types of arachidonic acid-containing molecules, anandamide (N-arachidonoylthanolamine) and 2-AG (Fig. 1), have been identified as the endogenous ligands for the cannabinoid receptors. The first endogenous cannabinoid receptor ligand to be found was anandamide. This molecule was isolated from pig brain by Devane et al. (6) in 1992. Anandamide binds to both the central and peripheral cannabinoid receptors and induces a variety of cannabimimetic activities such as the inhibition of mouse twitch response, reduction of spontaneous motor activities, immobility, hypothermia, analgesia, impairment of memory, and the inhibition of long-term potentiation (7). Despite the diverse pharmacological activities of anandamide mentioned above, there are several enigmas concerning the physiological significance of anandamide in vivo. 1) The tissue levels of anandamide are usually very low. For example, the level of anandamide in rat brain was reported to be 3 – 15 pmol/g tissue and that in mouse brain was 10 – 15 pmol/g tissue (8 – 10). In fact, the N-arachidonoyl species (anandamide) is usually a minor species of N-acylthanolamines present in various mammalian tissues (8, 9). 2) It has been demonstrated that anandamide can be formed through several metabolic pathways (8 – 10). However, no selective and efficient synthetic pathway for anandamide has hitherto been found. 3) Anandamide acts as a partial agonist toward the cannabinoid receptors in many cases (9). In particular, anandamide is a weak partial agonist of the CB\textsubscript{2} receptor. It is curious that an endogenous natural ligand acts as a partial agonist toward its own receptor; it seems unlikely, therefore, that anandamide acts as an endogenous CB\textsubscript{1} and CB\textsubscript{2}-receptor agonist with significant physiological importance. In addition to acting as an endogenous ligand for these cannabinoid receptors (CB\textsubscript{1} and CB\textsubscript{2}), anandamide has been shown to act as an agonist toward other receptors such as an ion channel-type vanilloid receptor (VR\textsubscript{1} receptor). It is conceivable that some of the anandamide effects mentioned above are mediated through receptors or binding sites other than the CB\textsubscript{1} and CB\textsubscript{2} receptors.

The second endogenous cannabinoid receptor ligand to be found was 2-AG, a unique molecular species of 2-monoacylglycerol containing arachidonic acid. We isolated 2-AG from rat brain (11) and Mechoulam et al. (12) isolated it from canine gut. 2-AG binds to the cannabinoid receptors with high affinity, although its affinity was somewhat lower than that of anandamide (9, 13). Like anandamide, 2-AG exhibits various pharmacological activities in vitro and in vivo, yet it is
not clear in some cases whether these effects of 2-AG are actually mediated through the cannabinoid receptors or due to arachidonic acid metabolites that may have been derived from 2-AG.

As for 2-AG, there are several notable issues. 1) The tissue levels of 2-AG are usually markedly higher than those of anandamide in the same tissue. For example, the amount of 2-AG in rat brain was reported to be 0.23 – 4.0 nmol/g tissue, which is tens to hundreds times higher than that of anandamide (9, 13). 2) There are several metabolic pathways responsible for the generation of 2-AG. First of all, 2-AG can be formed from inositol phospholipids through the combined actions of phospholipase C and diacylglycerol lipase or through the combined actions of phospholipase C and phospholipase A\(_1\) and phospholipase C (Fig. 2) (9, 13). The first pathway, involving the rapid hydrolysis of phospholipids by phospholipase C and subsequent hydrolysis of the resultant diacylglycerol by diacylglycerol lipase, was described as a degradation pathway for arachidonic acid-containing diacylglycerols in platelets (14). Stella et al. (15) demonstrated that these enzyme activities are involved in the ionomycin-induced generation of 2-AG in cultured neurons. 2-AG can also be formed from arachidonic acid-containing phosphatidylcholine through the action of phospholipase C or the combined actions of phospholipase D and phosphatidic acid phosphatase (Fig. 2). Alternative pathways for the generation of 2-AG are the conversion of 2-arachidonoyl LPA to 2-AG and the conversion of 2-arachidonoyl PA to 2-AG (Fig. 2). The biosynthetic pathways for 2-AG appear to differ, depending on the types of tissues and cells and the types of stimuli. 3) 2-AG acts as a full agonist toward either the CB\(_1\) receptor or the CB\(_2\) receptor. Previously, we found that 2-AG induces a rapid transient increase in the intracellular free Ca\(^{2+}\) concentrations ([Ca\(^{2+}\)]\(_i\)) in neuroblastoma \(\times\) glioma hybrid NG108-15 cells which express the CB\(_1\) receptor (16, 17). We confirmed that the response induced by 2-AG was blocked by pretreatment of the cells with SR141716A (Fig. 1), a CB\(_1\) receptor-specific antagonist, indicating that the response induced by 2-AG was mediated through the CB\(_1\) receptor (18). We also found that similar 2-AG-induced Ca\(^{2+}\) transients are observed in promyelocytic HL-60 cells, which express the CB\(_2\) receptor. We confirmed that the response was abolished by pretreatment of the cells with SR144528 (Fig. 1), a CB\(_2\)-receptor-specific antagonist, indicating that the response induced by 2-AG was also mediated through the CB\(_2\) receptor (18). The structure-activity relationship of 2-AG and other cannabinoid receptor ligands was then investigated in detail using this assay system. Anandamide was found to act as a weak partial agonist toward both the CB\(_1\) and CB\(_2\) receptors. The activity of \(\Delta^+\)-THC, a major psychoactive constituent of marijuana, was also low. We further examined the activities of various 2-AG analogs. The activities of monoacylglycerols containing various saturated, monoenoic, and dienoic fatty acids were very low. Among various naturally occurring analogs, 2-AG acted as the most efficacious agonist toward both types of receptors (CB\(_1\) and CB\(_2\)). Several investigators have also demonstrated that 2-AG is the most efficacious agonist among various 2-AG and anandamide analogs (19 – 21). Based on these experimental results, we have proposed that 2-AG rather than anandamide is the true natural ligand for the cannabinoid receptors (17, 18).

Recently, the occurrence of several novel analogs of anandamide, that is, virodhamine (\(O\)-arachidonoyl-ethanolamine) and \(N\)-arachidonoyldopamine, in mammalian tissues has been reported by several investigators (22, 23). The levels of these novel analogs of anandamide in living tissues are, however, generally very low; it is not clear at present whether these anandamide analogs, virodhamine and \(N\)-arachidonoyldopamine, actually play specific physiological roles as signaling molecules in living animals. An ether-linked analog of 2-AG (2-AG ether or noladin ether) has also been reported to occur in pig (24) and rat (25) brains. However, the amounts of 2-AG ether (noladin ether) in the brains of several mammalian species were found to be negligible, if at all present (26). It is thus premature to regard 2-AG ether (noladin ether) as an endogenous cannabinoid receptor ligand.

![Fig. 2. Pathways involved in the synthesis and degradation of 2-AG. PC, phosphatidylcholine; PI, phosphatidylinositol; PIP\(_2\), phosphatidylinositol bisphosphate; PA, phosphatidic acid; DG, diacylglycerol; MG, monoacylglycerol; AA, arachidonic acid; LPA, lysophosphatidic acid; PLC, phospholipase C; PLD, phospholipase D; PLA\(_1\), phospholipase A\(_1\).](image-url)
Expression of the cannabinoid receptors in leukocytes

Cannabinoid receptors are present abundantly in certain areas of lymphoid tissues such as the marginal zone of the spleen, the cortex of the lymph nodes and the nodular corona of Peyer's patches (4, 27). The predominant cannabinoid receptor expressed in these lymphoid tissues is the CB2 receptor. It is noteworthy that the levels of the CB2 receptor mRNA present in spleen and tonsil are almost equivalent to that of the CB1 receptor mRNA in the central nervous system. On the other hand, specific receptor binding was absent in T lymphocyte-enriched areas such as the thymus and periartheriolar lymphatic sheaths of the spleen. CB2 receptor mRNA is expressed in various types of leukocytes with a rank order of B lymphocytes > natural killer cells > monocytes/macrophages > polymorphonuclear leukocytes > T8 lymphocytes > T4 lymphocytes (28). Mast cells (29), microglia cells (30) and dendritic cells (31) have also been shown to contain the CB2 receptor mRNA. We also found that the CB2 receptor mRNA is abundantly expressed in human peripheral blood eosinophils (32). Interestingly, human neutrophils did not contain a detectable amount of the CB2 receptor mRNA; it seems possible that the CB2 receptor mRNA detected in polymorphonuclear leukocytes (28) is attributable mainly to that expressed in eosinophils but not neutrophils.

What is the physiological function of the CB2 receptor? The expression of the CB2 receptor is restricted almost exclusively to several types of inflammatory cells and immunocompetent cells mentioned above. This strongly suggests that the CB2 receptor plays some essential roles during the course of inflammatory reactions and immune responses. Currently, we have several lines of evidence that support this notion. Our recent experimental results showing that the CB2 receptor and 2-AG play essential stimulative roles in inflammation are described below.

2-AG induces the activation of mitogen-activated protein kinases (MAP kinases)

MAP kinases are key enzymes in the intracellular signal transduction in diverse cell types. We therefore examined the effects of 2-AG on MAP kinases in HL-60 cells. We found that 2-AG induces rapid phosphorylation and activation of p42/44 MAP kinase in HL-60 cells (Fig. 3) (33). The addition of SR144528, a CB2-receptor-specific antagonist, to the cells prior to the addition of 2-AG, indicating that the CB2 receptor is involved in the response. Gq/11 is also assumed to be involved, because pertussis toxin (PTX) treatment of the cells abrogated the response induced by 2-AG. CP55940 and anandamide also induced the activation of p42/44 MAP kinase, although the activation by anandamide was less pronounced than that by 2-AG or CP55940. Similar rapid 2-AG-induced activation of p42/44 MAP kinase was observed with other types of cells such as human eosinophilic leukemia EoL-1 cells (S. Oka and T. Sugiura, unpublished results).

What then is the physiological meaning of the 2-AG-induced activation of p42/44 MAP kinase? It is well known that p42/44 MAP kinase phosphorylates transcription factors in the nucleus, thereby regulating gene transcription. The major nuclear target of p42/44 MAP kinase is the Elk-1 protein. The reaction product, phosphorylated Elk-1, then activates transcription of the c-fos promoter through a ternary complex assembled on the c-fos serum response element. Such activation of immediate response genes is known to be essential for various cellular responses. It is possible that 2-AG, like various growth factors, induces the activation of gene transcription through sequential phosphorylation and activation of p42/44 MAP kinase and transcription factors such as Elk-1 to elicit cellular responses. Recently, we obtained evidence that p42/44 MAP kinase plays crucial roles in 2-AG-induced augmented production of chemokines and 2-AG-induced cell migration as described below.

Notably, 2-AG provoked not only the activation of the p42/44 MAP kinase but also the activation of p38 MAP kinase and c-Jun N-terminal kinase (JNK) in HL-60 cells (34). 2-AG-induced activation of p38 MAP kinase...
kinase and JNK has also been reported by several investigators for other cell types (35, 36). It is noteworthy that various pro-inflammatory molecules are known to induce the activation of p38 MAP kinase and JNK (37). It seems possible that 2-AG, like other pro-inflammatory molecules, induces the activation of p38 MAP kinase and JNK in addition to p42/44 MAP kinase in leukocytes, thereby stimulating inflammatory reactions.

2-AG induces augmented production of pro-inflammatory chemokines

We next examined the effects of 2-AG on the production of pro-inflammatory chemokines in human promyelocytic leukemia HL-60 cells. We found that 2-AG induces a marked acceleration in the production of interleukin 8 (IL-8) and macrophage-chemotactic protein-1 (MCP-1) (38). The effect of 2-AG was blocked by treatment of the cells with SR144528, indicating that the effect of 2-AG is mediated through the CB2 receptor. p42/44 MAP kinase and MEK (MAP kinase kinase) are also assumed to be involved, because treatment of the cells with PD98059 (a MEK inhibitor) reduced the production of IL-8. Notably, not only 2-AG but also CP55940 accelerated the production of IL-8 and MCP-1, whereas anandamide failed to augment the production of chemokines. Jbilo et al. (39) also demonstrated the enhanced production of chemokines such as IL-8 and MCP-1 in CP55940-stimulated HL-60 cells. As for other types of cytokines, several investigators reported that 2-AG induces the inhibition of the production of IL-6 in J774 macrophage-like cells (40) and the inhibition of TNF-α production in lipopolysaccharide (LPS)-stimulated mouse macrophages (41). These results may suggest that 2-AG plays suppressive roles in inflammatory reactions and immune responses. This appears to be rather contradictory to our experimental results mentioned above. A possible reason for this discrepancy may be a difference in the responses and cytokines in our studies and those of other investigators. Another possibility is that arachidonic acid and/or its metabolites that may have been formed from 2-AG are more or less implicated in the responses induced by 2-AG. We confirmed that arachidonic acid did not induce augmented production of chemokines (38). Hence, arachidonic acid and/or its metabolites that may have been derived from 2-AG are not implicated in our cases.

Interestingly, 2-AG and lipopolysaccharides (LPS) acted synergistically to induce the markedly augmented production of IL-8 in HL-60 cells differentiated into macrophage-like cells (Table 1) (38). 2-AG may play an important role in the acceleration of chemokine production in leukocytes stimulated with pro-inflammatory molecules.

2-AG induces rapid actin rearrangement and morphological changes

The effects of 2-AG on the morphology as well as the actin filament system in differentiated HL-60 cells, which express the CB2 receptor, were next examined. We found that 2-AG induces rapid morphological changes such as the extension of pseudopods (42). We also found that 2-AG provokes a rapid actin polymerization in these cells (42). The actin polymerization induced by 2-AG reached a peak 10 s after the addition of 2-AG and returned to the basal level after 1 min. CP55940 possessed activity comparable to that of 2-AG. WIN55212-2, a cannabimimetic aminoalkylindole, also possessed appreciable activity similar to that of CP55940, whereas WIN55212-3, an inactive isomer of WIN55212-2, failed to induce actin polymerization. Free arachidonic acid and anandamide also did not exhibit any activity. The actin polymerization induced by 2-AG was abolished when cells were treated with SR144528 and PTX, suggesting that the response was mediated by the CB2 receptor and G_{i/o}. A phosphatidylinositols 3-kinase, Rho family small G proteins, and a tyrosine kinase were also suggested to be involved. Reorganization of the actin filament system is known to be indispensable for a variety of cellular processes such as cell motility. It is plausible that 2-AG plays physiologically essential roles in various inflammatory cells and immunocompetent cells by inducing rapid actin rearrangement.

2-AG induces the migration of inflammatory cells

We then examined the effects of 2-AG on the motility

| Table 1. Effects of 2-AG and LPS on the production of IL-8 in HL-60 cells differentiated into macrophage-like cells |
|--------------------------------------------------|-----------------|------------------------------|-----------------|
| DMSO | 2-AG | LPS + DMSO | LPS + 2-AG |
| ng/ml | | | |
| 5.3 ± 0.3 | 11.0 ± 3.2* | 74.6 ± 4.8** | 98.6 ± 2.5** |
of HL-60 cells differentiated into macrophage-like cells. We found that 2-AG induces the migration of differentiated HL-60 cells (43). The proportion of migrated cells was 3.4% for the control and 26.7% for the 2-AG (1 μM)-stimulated cells following 4-h incubation in the Transwell™ inserts. The number of migrating cells increased dose-dependently. The effect was observed from 10 nM 2-AG and reached a peak at 10 μM. The EC₅₀ was around 430 nM. The migration induced by 2-AG was blocked by treatment of the cells with either SR144528 or PTX (Fig. 4), suggesting that the CB₂ receptor and Gi/o are involved in the 2-AG-induced migration. Several intracellular signaling molecules, such as Rho kinase, p42/44 MAP kinase and p38 MAP kinase, were also suggested to be involved. In contrast to 2-AG, anandamide did not induce the migration. We then examined whether the 2-AG-induced migration is the result of chemotaxis or chemokinesis. In this experiment, we employed 2-AG ether (a stable ether-linked analog of 2-AG) instead of 2-AG. We found that 2-AG ether elicits mainly chemotaxis rather than chemokinesis; it is likely that 2-AG also induces mainly chemotaxis. The 2-AG-induced migration was also observed for two other types of macrophage-like cells (U937 cells and THP-1 cells) (43), human peripheral blood monocytes (43), human peripheral blood eosinophils (32), human eosinophilic leukemia EoL-1 cells (32), human natural killer leukemia KHYG-1 cells (S. Kishimoto and T. Sugiura, unpublished results), and human peripheral blood natural killer cells (S. Kishimoto and T. Sugiura, unpublished results). Several investigators have also demonstrated that 2-AG induces the migration of mouse splenocytes (44) and microglia cells (45). These results strongly suggest that 2-AG induces the migration of several types of leukocytes through a CB₂-receptor-dependent mechanism, thereby stimulating inflammatory reactions and immune responses.

Evidence that 2-AG is involved in inflammatory reactions in vivo

Finally, we examined whether the CB₂ receptor and 2-AG are involved in inflammation in vivo. First, we investigated possible implications of the CB₂ receptor and 2-AG in acute inflammation in mouse ear induced by the topical application of 12-O-tetradecanoylphorbol 13-acetate (TPA). We found that the amount of 2-AG in mouse ear is markedly augmented following the application of TPA with a concomitant increase in ear thickness (S. Oka, S. Yanagimoto, and T. Sugiura, unpublished results). In contrast to 2-AG, the amount of anandamide did not change markedly. Importantly, TPA-induced ear swelling was blocked by treatment of the ear with SR144528 but not with AM251, a CB₁-receptor antagonist, suggesting that the CB₂ receptor rather than the CB₁ receptor is involved in TPA-induced ear swelling.

The application of SR144528 reduced not only ear swelling but also the TPA-induced leukotriene B₂ production and the infiltration of neutrophils into mouse ear. Interestingly, the topical application of 2-AG to mouse ear evoked ear swelling, which was abolished by treatment of the ear with SR144528 but not with AM251. These results strongly suggest that the CB₂ receptor and 2-AG play crucial stimulative roles during the course of acute inflammation induced by TPA.

We next investigated possible involvement of the CB₂ receptor and 2-AG in delayed-type hypersensitivity. In this experiment, we examined oxazolone-induced allergic inflammation in mouse ear and employed mice that had been sensitized with oxazolone 5 days before the challenge. We found that the level of 2-AG in mouse ear is markedly augmented following the challenge with oxazolone (S. Oka, S. Ikeda, and T. Sugiura, unpublished results). In contrast to 2-AG, the amount of anandamide remained unchanged. Notably, the ear swelling induced by oxazolone was markedly reduced by the topical application of SR144528. In contrast, the effect of AM251, a CB₁-receptor antagonist, was marginal, indicating that the CB₂ receptor rather than the CB₁ receptor is involved in oxazolone-induced allergic inflammation as in the case of TPA-induced acute inflammation described above.

In addition to this, recently we obtained evidence that
2-AG is implicated in carrageenan-evoked peritonitis in mice (S. Oka, S. Yanagimoto, and T. Sugiura, unpublished results). Iwamura et al. (46) also reported that several CB2 receptor antagonists reduced carrageenan-induced rat paw edema. It is conceivable, therefore, that close involvement of the CB2 receptor and 2-AG is a common feature of various types of inflammatory reactions in vivo.

Not much is known concerning the physiological and pathophysiological roles of the CB2 receptor in inflammation in vivo. Several investigators reported that Δ⁹-THC and the CB2 receptor exert suppressive effects on inflammatory reactions and immune responses. For example, it has been reported that the oral administration of Δ⁹-THC suppressed rat paw edema induced by carrageenan (47). We also found that the topical application of Δ⁹-THC reduced mouse ear swelling induced by TPA (S. Oka, S. Yanagimoto, and T. Sugiura, unpublished results). These inhibitory effects of Δ⁹-THC are not at variance with the idea that the endogenous CB2-receptor agonist, that is, 2-AG, plays important stimulative roles in inflammation because it has already been demonstrated that Δ⁹-THC acts as an antagonist toward the CB2 receptor (48). It is plausible that Δ⁹-THC counteracts the effect of 2-AG at the CB2 receptor as in the case of SR144528, thereby reducing inflammatory reactions.

There are also several reports showing that certain cannabinoid receptor agonists, such as WIN55212-2, exert inhibitory effects on inflammatory reactions in vivo (49 – 52). The detailed mechanism underlying this is yet to be clarified. A possible explanation is that synthetic cannabinoid receptor agonists interfere with the action of the physiological ligand 2-AG. Synthetic ligands, such as WIN55212-2, are metabolically rather stable and, due to this, the anergic condition, which is attributed to desensitization and subsequent receptor-internalization, is sustained for longer periods compared with the case of 2-AG. We confirmed this using HL-60 cells desensitized with WIN55212-2 or 2-AG. Such a prolonged anergic condition induced by synthetic cannabinoid receptor agonists may provide a reason why these compounds interfere with the action of 2-AG and behaves almost as an antagonist in some cases in vivo; however, additional studies are necessary to draw a conclusion. In any event, it appears that these previous experimental results do not conflict with the notion that the CB2 receptor and 2-AG play important roles in stimulating the inflammatory reactions mentioned above.

**Concluding remarks**

The CB2 receptor is abundantly expressed in several types of inflammatory cells and immunocompetent cells and has been assumed to play an essential role in inflammatory reactions and immune responses. Nevertheless, its physiological function in vivo still remains an enigma. In particular, little information has thus far been available concerning possible implications of the CB2 receptor and 2-AG in inflammation. As described in this review, we have explored possible physiological roles of the CB2 receptor and 2-AG in inflammation. Based on our experimental results and those by others, we assume that the physiological roles of 2-AG in inflammatory reactions are as follows: Various types of inflammatory cells and immunocompetent cells, such as macrophages, generate 2-AG when these cells are stimulated and release 2-AG into the extracellular fluid (53 – 56). The released 2-AG then binds to the CB2 receptor expressed on other inflammatory cells or immunocompetent cells, induces the augmented production of chemokines such as IL-8 that induce the infiltration of neutrophils, and triggers direct migration of macrophages, eosinophils and natural killer cells, thereby stimulating inflammatory reactions (Fig. 5). On the other hand, Δ⁹-THC and SR144528, CB2-receptor antagonists, interfere with the actions of the CB2 receptor and its physiological ligand 2-AG, thereby causing suppression of inflammatory reactions. Whether this scheme is true or not will be determined in the near future. In any case, it is apparent that 2-AG plays an important part during the course of a variety of inflammatory reactions, such as acute inflammation and allergic inflammation in vivo, yet the details of the mechanism as well as the mode of action of 2-AG
remain to be clarified. Further detailed studies on the CB₂ receptor and 2-AG are essential for a thorough elucidation of the precise regulatory mechanisms of various inflammatory reactions.

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