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REVIEW ARTICLE

Cannabinomimetic Control of Mast Cell Mediator Release: New Perspective in Chronic Inflammation

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The present review aims to elucidate the emerging role played by cannabinomimetic compounds in the control of mast cell activation. Mast cells are immune competent cells strategically localised at the sites directly interfacing with the external environment, which, in case of injury, regulate the immune response by the release of a plethora of both pre-formed and newly-synthesised mediators. However, although the main goal of mast cell activation is to initiate the inflammatory reaction, and thus maintain internal homeostasis, the consequences of dysregulated mast cell activation could be to chronically activate the inflammatory response as occurs in arthritis, inflammatory bowel diseases, atherosclerosis and asthma. Therefore, much effort has been made to develop compounds that act to prevent mast cell degranulation. Cannabinomimetic compounds (i.e. agents able to modulate endocannabinoid function) are considered as an emerging class of regulators of mast cell behaviour. We focus on the evidence for a cannabinomimetic control of both acute and chronic inflammatory disease, recognising a common mast cell origin for problems such as dermatitis, inflammatory gastrointestinal syndrome and granuloma formation. Special emphasis is provided for the recent promising results obtained with palmitoylethanolamide in human studies. In the light of evidence suggesting that the control of mast cell activation at an early time during an inflammatory process may account for its resolution, it is reasonable to propose that cannabinomimetic compounds, including palmitoylethanolamide and its congeners, could represent possible candidates for treating several chronic inflammatory diseases.

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Mast cells: a brief overview

Mast cells (MCs) were first described in 1879 by Paul Ehrlich due to their metachromatic properties that depend upon the presence and the degree of sulphatation of proteoglycans, such as heparin. Progenitors of mature MCs are multipotent haematopoietic stem cells within the bone marrow identified by positive immunostaining for CD34 and c-kit (but negative for FcεRI) (1). CD34 and c-kit positive cells leave the bone marrow, circulate in the blood and, depending upon the presence of specific chemotactic signals, migrate to the tissue where they become resident. MC precursors proliferate and differentiate into the tissue depending on to the presence of local growth factors and cytokines, such as stem cell factor (SCF), interleukin (IL)-3, IL-4, IL-4, IL-9 and neural growth factor (NGF) (2), secreted both by the MC precursors themselves and by other tissue cells.

Originally, in rodents, MCs were divided into two subtypes depending on the variable content of the neutral serine proteases, tryptase and chymase. Thus, MCs containing only tryptase represent the mucosal MCs, preferentially localised in the mucosa of the airways and gastrointestinal tract. MCs containing chymase, tryptase, carbossipeptidase and chatepsin G are defined as connective-tissue MCs, which are typically found in the skin, synovium, peritoneum and perivascular tissues. However, in other species, including humans, another subtype has been identified containing chymase and carbossipeptidase and possessing a different tissue localisation (3).

Due to their strategic localisation at sites directly interfacing with the external environment, MCs act as surveillance antennae

against different types of injury and, in times of need, can activate and regulate both innate and adaptive immune mechanisms (3). Moreover, MCs possess important physiological roles that support homeostatic control, such as in tissue remodelling, wound healing, and the neuroimmune response to stress (4) and are located in close proximity to blood and lymphatic vessels (5) and nerves (6) in order to perform this function.

MCs possess several biological mediators that are released from cytoplasmic granules primarily due to stimulus-induced degranulation; including vasoactive amines such as histamine, proteoglycans (mainly heparin and chondroitin sulphate), neutral serine proteases, such as tryptase and chymase, cytokines and growth factors such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor, NGF, transforming growth factor (TGF)-β and tumour necrosis factor alpha (TNF-α) (4). However, activated MCs can also induce *de novo* synthesis of several inducible enzymes, such as inducible nitric oxide synthase (NOS) or cyclo-oxygenase-2 (and their products nitric oxide, prostaglandins and leukotrienes), together with a plethora of chemokines and cytokines, TNF-α, IL-1α, IL-1β, IL-6, IL-9, IL-12, IL-18 and TGF-β, which function to amplify the inflammatory/immune reaction.

MC activation occurs both via an immunoglobulin (Ig)E-dependent explosive degranulating (anaphylactic) manner as well part of a more controlled secretory process in response to non-IgE-related stimuli (7) (e.g. bacterial or viral infection), or in response to certain drugs such as compound 48/80, ionomycin and opioids (8). Interestingly, it has been discovered that MCs not only respond to exogenous stimuli, but also to various endogenous hormones and neuropeptides that exert a fine control over degranulation. For example, oestradiol enhances IgE-dependent mast cell activation, resulting in a shift of the allergen dose response and, more generally, sex hormones can influence (directly or indirectly) the number and activation state of MCs (e.g. via oestrogen receptors that are present on peritoneal MCs in rats) (9). Additionally, during neuronal inflammation, MCs may respond to neuropeptides such as substance P, NGF or calcitonin gene-related peptide and cardiac MCs have been shown to respond to atrial natriuretic peptide, which could induce adverse ventricular remodelling (10).

It has also been reported that tissue MC activation during disease progression may occur without degranulation (11). Recently IL-1 and TNF-α were found to induce secretion of cytokines from MCs without affecting histamine release (11). Because physiological and pharmacological compounds are able to activate MCs in a different manner, different mechanisms of MC activation can be hypothesised to exist, ranging from the selective secretion of an individual class of cytokines or amines, as observed in anaphylaxis, to an exhaustive degranulation and acute secretion of a plethora of mediators during other pathologies.

Mast cells in inflammation

MCs have long been known to participate in the inflammatory process and this knowledge was based on the evidence that MCs are present and recruited to all inflammatory sites, from lesions of patients with Crohn's disease to the synovium of patients suffering with rheumatoid arthritis. However, it has only recently become clear that MCs play an important role in orchestrating the whole inflammatory process from initiation events to chronic activation. MCs are the first immune cells to considerably stimulate the inflammatory process due to a rapid release of pro-inflammatory and vasoactive mediators. In particular, histamine, cytokines and proteases facilitate a pro-inflammatory microvascular environment due to increased vasodilatation, increased vascular permeability and local oedema formation, thus increasing the tissue delivery of other pro-inflammatory cells.

Moreover, histamine and TNF-α, released by MCs, increase inflammatory cell recruitment due to an increase in (i) endothelial adhesion molecules expression (selectin and integrins), and thus the stimulation of leucocyte endothelial adhesion and rolling, and (ii) MC-derived leukotrienes and IL-8, which act as chemotactic signals for eosinophils, neutrophils and basophils (12). Therefore, even if the main goal of MC activation is to initiate the inflammatory response to maintain internal homeostasis, excessive MC activation could lead to unwanted chronic inflammation.

Our previous studies demonstrate the role of MCs in the chronic inflammatory process, showing that granuloma formation in rats was accompanied by massive mast cell degranulation, whereas the depletion of MC granule contents reduced granulomatous tissue formation by more than 50% (13). These data are in accordance with other studies showing that mice with a mast cell deficiency are less likely to develop chronic inflammatory conditions, such as autoimmune encephalomyelitis and arthritis, whereas the induction of these inflammatory conditions and the severity of symptoms were increased after restoration of the mast cell population (14).

Moreover, we showed that treatment with ketotifen, a mast cell (MC) membrane stabiliser, if given at a sufficient time point (at the sponge implantation time) was able to control the subsequent chronic inflammatory process, confirming that MCs orchestrate the initiation of the inflammatory reaction due to the release of preformed mediators.

The central role of mast cell mediators in inflammation was studied not only in animal models, but also in human inflammatory diseases and rheumatic diseases (15). Recent studies focussed on the role of MC-released chymase in coronary inflammation (16) or VEGF in asthma (17) and NGF in atopic dermatitis and psoriasis (18). Interestingly, many of these pathologies are exacerbated by stress. Although it is not well established whether MCs are the root cause, there is clear evidence of a bidirectional communication between MCs and the nervous system. This connection is well studied during the aethiopathogenesis of irritable bowel disease (IBD); for example using colonic biopsies from patients suffering from IBD, MCs have been found to be in close contact with nerves and to activate them via mediator release (7). Nevertheless, it is well known that stress could initiate MC degranulation and, in this way, start or exacerbate IBD symptoms (19).

Control of mast cell activation

The importance of MC degranulation during inflammation has led to a concerted effort to develop pharmaceutical compounds that

act to prevent MC activation. To date, many drugs have been produced that inhibit mast cell activation and mediator release; among these drugs, the first to be used clinically were the classical anti-H1 histamine receptor antagonists, which act to block the adverse effects of histamine and were shown to inhibit mast cell activation in *in vitro* studies (20). The MC membrane stabilisers such as cromolyn and ketotifen have been successfully used in paediatric allergic disorders. In addition, tranilast, an anti-allergic drug used in the treatment of bronchial asthma, atopic dermatitis and allergic conjunctivitis, has recently been shown to possess anti-angiogenic properties via inhibition of mast cell-derived chymase and TGF-β (21). Recent drug-based approaches to MC control include the protease inhibitors such as tryptase inhibitors (APC 2059) used in the treatment of asthma and ulcerative colitis (22), and several orally active chymase inhibitors used in certain cardiovascular disease states, such as atherosclerotic lesions (16). Moreover, drugs targeting SCF and/or c-kit which are necessary for mast cell proliferation and survival, such as anti-SCF antibodies or antisense oligonucleotides, have been studied in allergic inflammation (23). Anti-inflammatory therapies aimed at either reducing MC numbers or stabilising the existing MC population are now considered one of the best avenues to follow. Interestingly, the cannabinoids and relative cannabinomimetic compounds are an emerging class of compounds that dampen the inflammatory response via a modulation of mast cell activation.

Natural and synthetic cannabinoids and their receptors

The term cannabinoids (CBs) refers to the biologically active components of the *Cannabis sativa* plant. In the 19th century, marijuana was prescribed by physicians for the treatment of diseases such as inflammation (arthritis), infections (gonorrhoea), pain (relief from tooth pain), eating disorders (appetite stimulants), and as muscle relaxants (24). However, since the potential pshychotropic effects of marijuana were discovered, its potential therapeutic use has been limited. The main biologically active component of marijuana is Δ^9-tetrahydrocannabinol (THC) which has been extensively studied both *in vitro* and *in vivo* (25). However, a further constituent of marijuana is cannabidiol, which produces the same biological effects as Δ^9-THC, but is less likely to induce psychotropic side effects (26).

The biological effects of CBs are determined via their binding to two cell membrane G_0-protein coupled receptors, named CB_1 and CB_2. Previously CB_1 was also referred to as the 'central' cannabinoid receptor because it is mainly expressed in the central nervous system, and CB_2 was referred to as the 'peripheral' cannabinoid receptors because they were predominantly found on immune cells (24). It is now well recognised that both receptor subtypes are ubiquitously expressed in many cell types, including both the central nervous system and peripheral tissues. It appears that CB_1 within specific sites in the central nervous system are responsible for the psychotropic side effects of CB usage.

Synthetic analogues of Δ^9-THC have been developed, such as CP55,940 or WIN55,212-2 which are high affinity ligands for both CB_1 and CB_2,, ACEA or JWH-015 which are selective ligands for CB_1 and CB_2 respectively (27). Finally, selective receptor antagonists for CB_1, such as SR141716A (also described as an inverse agonist), and for CB2, such as SR144528, have also been developed which act to inhibit or reverse the biological effects exerted by CB_1 and CB_2 agonists.

Endogenous cannabinoids and the endocannabinoid system

The discovery of CB receptors was followed by the demonstration that mammalian tissue can produce endogenous agonists for these receptors, namely endocannabinoids. Endocannabinoids are all produced via enzymatic cleavage of membrane fatty acid precursors (28), and each has different specificities for the various CB receptors. The first endocannabinoid to be discovered (29) and subsequently the most studied is *N*-arachidonoyl-ethanolamine (anandamide, AEA). AEA (the ethanolamide derivative of arachidonic acid) has a higher affinity for CB_1 than CB_2 and also binds to the so-called vanilloid receptor-type 1 (TRPV1) (30). Other endocannabinoids discovered include 2-arachidonoyl glycerol (2-AG), which acts as a full agonist for CB_2 and, 2-arachidonoylglyceryl ether (noladin ether) which exhibits higher affinity for CB_1. Recently, a new endocannabinoid-like compound, palmithoylethanolamide (PEA) has been isolated from mammalian and vegeatable tissues which does not bind to either CB_1 or CB_2. All endocannabinoids are synthesised 'on demand' by cleavage of membrane lipid precursors. Endocannabinoids together with their respective receptors and enzymes involved in CB synthesis and breakdown are referred as the 'endocannabinoid system' (ECS). There is evidence that AEA and 2-AG enter cells via a combination of simple diffusion and facilitated, carrier-mediated transport (31) where they perform their physiological role and are then metabolised by specific intracellular enzymes. AEA is metabolised by fatty acid amide hydrolase (FAAH) and 2-AG mainly by monoacylglycerol lipase (32). Theoretically, pharmaceutical compounds can be generated that target any aspect of the ECS, from endocannabinoid synthesis, release, membrane transport and enzymatic degradation. There is evidence that modulation of the ECS may occur naturally due to the co-release of endogenous fatty acid derivatives, such as PEA, which act to potentiate the effects of AEA and 2-palmitoyl glycerol (32). Studies have shown that PEA co-released with AEA has an 'entourage effect', which leads to an increased sensitivity of CB_1 or TPRV1 for AEA possibly via an allosteric interaction with the receptor or via some other undetermined mechanism (33). At present, the multiple fatty acid amine/amide derivatives, such as PEA, are all considered to be 'cannabinomimetic compounds', since they all modulate ECS function.

Cannabinoids and inflammation

It has been reported that CBs profoundly affect the immune system. Evidence suggests that CB receptor agonists (natural and synthetic), as well as drugs that indirectly modulate the ECS, can induce the down-regulation of a number of cytokines, chemokines as well arachidonic acid metabolites and also reduce nitric oxide overproduction during an inflammatory insult. Therefore, it appears

that the ECS possesses a protective role both in acute and chronic inflammatory diseases. *In vitro* studies demonstrate that the synthetic CB, WIN55,212-2, reduces nitric oxide over-production and inducible NOS synthesis in lipopolysaccharide-stimulated macrophages (34), in glial cells (35), and in C6 cells treated with interferon-γ and HIV-Tat protein and also inhibits the generation of inflammatory mediators by astrocytes *in vivo* (35). These protective effects of cannabinomimetics were also demonstrated *in vivo* utilising animal models of acute inflammation such as cerulein-induced pancreatitis in rats (36), and chronic inflammation, such as neurodegenerative-associated inflammation in rats and mice (35), and granuloma formation in rats (27). However, it is important to note that we have only presented a small amount of evidence from the large, and sometimes controversial (35), number of studies that exist concerning the cannabinomimetic control of inflammation. In recent years, interest in the anti-inflammatory properties of cannabinomimetic compounds has focussed on the control of MC activation.

Cannabinoids and mast cells: a closed relation

Interest in the role of the ECS system in modulating MC function stemmed from the identification of the various components of the ECS (receptors, enzymes responsible for synthesis and degradation) within the MC itself. The first evidence that CB receptors were present on mast cell membranes came from Facci *et al.* (37) who reported that rat basophil leukaemia RBL2H3 cells, a mast cell cognate cell-line, express both the CB_2 gene and a functional CB_2 receptor protein that transduces a negative regulatory effect on MC activation. Although both PEA and AEA bind to the CB_2 receptor on RBL-2H3 cells, only PEA down-regulates MC activation *in vitro* (37). Subsequent studies from other groups established that CB_1 receptors were also present on RBL2H3 cells and that co-expression of CB_1 and CB_2 is not functionally redundant because these receptors mediate different signalling pathways (38). Some of the anti-inflammatory effects of CB_1 ligands may be due to an elevation of cAMP, which, in turn, causes suppression of MC degranulation (39), whereas CB_2 receptors are the predominant regulatory receptors for the extra cellular signal-regulated kinase, AKT, signalling pathway in RBL2H3 cells (38). The presence of CB receptors was found not only in mastocyte-like cells, such as RBL-2H3 cells, but also in purified peritoneal MCs (40), and evidence suggests that activation of these receptors in peritoneal MCs can regulate their behaviour. CBs inhibit immunological activation of guinea pig MCs *via* nitric oxide (41), and similar effects were also reported in experiments utilising rat peritoneal MCs (42) and human basophils (41). Although it was originally reported that the human MC-line, HMC-1, did not express a functional CB receptor under physiological conditions (31), our recent data demonstrate that JWH-015, a selective CB_2 agonist, was able to prevent MC activation isolated from woman affected by endometrial inflammation (43).

CBs have also been shown to influence MC function *in vivo*. The selective CB_2 receptor agonist, JWH-133, reduces MC-dependent oedema in response to compound 48/80 in mouse ear pinna (44). Moreover, natural and synthetic CBs have a protective effect both in acute and chronic inflammatory pathologies sustained by MC degranulation. The CB_2 selective agonist, HU308, reduced arachidonic acid-induced oedema in mice (45), and both the nonpsychotropic agonist, HU320, and cannabidiol were shown to inhibit murine collagen-induced arthritis (46), and, finally, both the selective CB_1 agonist, ACEA, and the CB_2 agonist JWH-015 reduced granuloma formation and associated angiogenesis in rats (27).

Recent studies have demonstrated the presence of a functional vanilloid receptor on MCs that could, at least in part, mediate some of non-CB receptor-dependent effect of cannabinomimetic compounds (30). Not all of the effects of CB on MCs are mediated through CB_1 and CB_2 receptor activation because there is evidence to suggest that Δ^8- and Δ^9-THC reduce histamine release from rat peritoneal MCs via a CB receptor-independent mechanism (40). In addition, cannabidiol, which unlike synthetic CBs does not bind to either CB_1 or CB_2 receptors, triggers the activation of MCs (47).

Endocannabinoids may also produce effects that are independent from CB receptor activation via a so called 'ALIA' mechanism or 'entourage effect', as first described by Facci *et al.* (37). The ALIA mechanism refers to the capability of saturated fatty acid ethanolamides, such as PEA, to behave as local autacoids capable of negatively modulating MC activation and preventing an inflammatory response.

Although PEA does not bind with high affinity to CB_1 (K_i = 23.8 mM) or CB_2 (K_i = 13.9 mM) receptors, it still maintains CB-like anti-inflammatory actions in several MC-mediated experimental models of inflammation; for example, PEA reduces 48/80 (44) and carrageenin-induced oedema formation (48).

Because MC also posses the CB membrane transporter and the intracellular enzymes involved in the breakdown of endocannabinoids, a potential mechanism for the CB-like actions of PEA could be that PEA may inhibit the CB transporter present in RBL-2H3 and in HMC-1 cells, therefore blocking AEA reuptake (49) and preventing intracellular AEA-degradation by FAAH. With these considerations in mind, PEA may be regarded as a *trait d'union* between the different mechanisms of action of the various cannabinomimetic compounds because PEA could activate CB_1 or CB_2 receptors on MC, directly at very high concentrations, or indirectly through the increase in endocannabinoid tone.

The efficacy demonstrated by PEA in various animal models, linked to its low side-effect profile, has led to the use of this compound and its analogues in a number of further animal studies and human clinical trials. A pilot study with PEA indicated that this drug was able to reduce eosinophilic granuloma and eosinophilic plaque generation in cats (50).

Recently, a new drug containing PEA has been approved by the Food and Drug Administration for the treatment of dermatitis; moreover, a recent pilot study aimed to assess the efficacy and safety of the twice daily application of a topical emulsion containing 2% Adelmidrol, a PEA analogue, in 20 paediatric patients suffering from atopic dermatitis. This study showed an 80% increase in symptom resolution (51) via the inhibition of NGF release from MCs.

Discussion and conclusions

The aim of the present review is to elucidate the emerging role played by cannabinomimetic compounds in the control of MC activation. In the recent past, natural and synthetic CBs have been shown to possess great efficacy in the treatment of several animal models of inflammatory diseases, particularly those recognising MC origin. In humans, the therapeutic use of CBs has been limited by their well known psychotropic side-effects and therefore, until now, it has only been possible to study the involvement of the ECS in certain human pathologies. Interestingly, AEA levels were found to be up-regulated in biopsies collected from patients with ulcerative colitis or in patients with diverticular diseases (36). These findings, supported by the notion that endocannabinoids are released 'on demand', suggest a possible role in the restoration of tissue homeostasis in certain pathological conditions. Additionally, the increased expression of CB_2 receptors in epithelial tissue during human IBD and up-regulated CB_2 receptor expression in women affected by endometritis (43) are regarded as part of the homeostatic changes leading to the resolution of the inflammatory response.

The fact that CBs have been shown to block MC activation has increased interest in these compounds as therapeutic agents, particularly in light of the newly-discovered role for MCs in the promotion, progression and chronicity of the whole inflammatory process. The possibility of utilising selective CB_2 receptor agonists that specifically target CB receptors expressed on MCs (and are therefore free from psychotropic side effects) would be of benefit in diseases, such as asthma. Further support for the use of CBs in the treatment of asthma comes from a study using asthmatic volunteers where cannabis has been shown to produce a direct bronchodilating effect.

The ability of PEA to control MC degranulation, via a CB_1/CB_2 independent mechanism, has paved the way for its therapeutic use in both animals and humans. The PEA-mediated stabilisation of MCs has proven to be useful in the treatment of atopic and irritative dermatitis.

In conclusion, we can hypothesise that cannabinomimetic compounds, including PEA and its congeners, act to control MC activation and degranulation early during the inflammatory response, thus leading to a swift resolution and preventing the development of chronic inflammatory disease.

Conflicts of interest

The authors have declared no conflicts of interest.

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