

REVIEW

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Cannabinoid-based drugs targeting CB₁ and TRPV1, the sympathetic nervous system, and arthritis

Torsten Lowin* and Rainer H. Straub

Abstract

Chronic inflammation in rheumatoid arthritis (RA) is accompanied by activation of the sympathetic nervous system, which can support the immune system to perpetuate inflammation. Several animal models of arthritis already demonstrated a profound influence of adrenergic signaling on the course of RA. Peripheral norepinephrine release from sympathetic terminals is controlled by cannabinoid receptor type 1 (CB₁), which is activated by two major endocannabinoids (ECs), arachidylethanolamine (anandamide) and 2-arachidonylglycerol. These ECs also modulate function of transient receptor potential channels (TRPs) located on sensory nerve fibers, which are abundant in arthritic synovial tissue. TRPs not only induce the sensation of pain but also support inflammation via secretion of pro-inflammatory neuropeptides. In addition, many cell types in synovial tissue express CB₁ and TRPs. In this review, we focus on CB₁ and transient receptor potential vanilloid 1 (TRPV1)-mediated effects on RA since most anti-inflammatory mechanisms induced by cannabinoids are attributed to cannabinoid receptor type 2 (CB₂) activation. We demonstrate how CB₁ agonism or antagonism can modulate arthritic disease. The concept of functional antagonism with continuous CB₁ activation is discussed. Since fatty acid amide hydrolase (FAAH) is a major EC-degrading enzyme, the therapeutic possibility of FAAH inhibition is studied. Finally, the therapeutic potential of ECs is examined since they interact with cannabinoid receptors and TRPs but do not produce central side effects.

Introduction

Rheumatoid arthritis (RA) is a debilitating disease that affects around 1.3 million people in the US alone [1]. Important characteristics of RA are inflammation of the joint with subsequent destruction of cartilage, pannus formation and infiltrates of immune cells [2–4]. Ongoing inflammation also leads to systemic changes manifesting in co-morbidities like dyslipidemia, depression, fatigue, insulin resistance, activation of the sympathetic nervous system, and cachexia [5, 6]. Changes in sympathetic activity lead to a metabolic switch, which is in part responsible for the perpetuation of inflammation and the increase in cardiovascular risk in RA patients [7].

Cannabis has been used since 4000 BC for the treatment of spasms and post-operative pain [8]. In the

1990s, the two main receptors for cannabinoids (cannabinoid receptors I and II; CB₁ and CB₂) were identified [9, 10]. Both receptors are activated by the psychoactive component of cannabis, tetrahydrocannabinol (THC), and several other synthetic and plant-derived cannabinoids [11]. Two major endogenous cannabinoids (endocannabinoids, ECs), arachidylethanolamine (anandamide, AEA) and 2-arachidonylglycerol (2-AG), were described shortly after the discovery of CB₁ and CB₂ [12, 13]. In recent years, other receptors such as transient receptor potential vanilloid 1 (TRPV1), GPR55, or GPR18 were found to bind cannabinoids, and activation of these receptors is responsible for the off-target effects of several cannabinoids [14–18]. Transient receptor potential channel (TRP) modulation by cannabinoids might be explicitly important since these receptors not only influence sensation of pain, but also support inflammation [19].

This review describes physiological aspects of CB₁ receptors, pharmacological roles of ECs and the EC-

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degrading enzyme fatty acid amid hydrolase (FAAH), functional crosstalk between ECs and TRPV1, the interaction between ECs and the sympathetic nervous system in RA, the influence of ECs on arthritis disease sequelae in mice and humans, and direct immunomodulatory effects of CB₁ signaling in the periphery and in the brain. Considering this knowledge we finally try to demonstrate an optimum therapeutic EC approach in RA.

Physiology

CB₁ influences cell function by controlling neurotransmitter levels

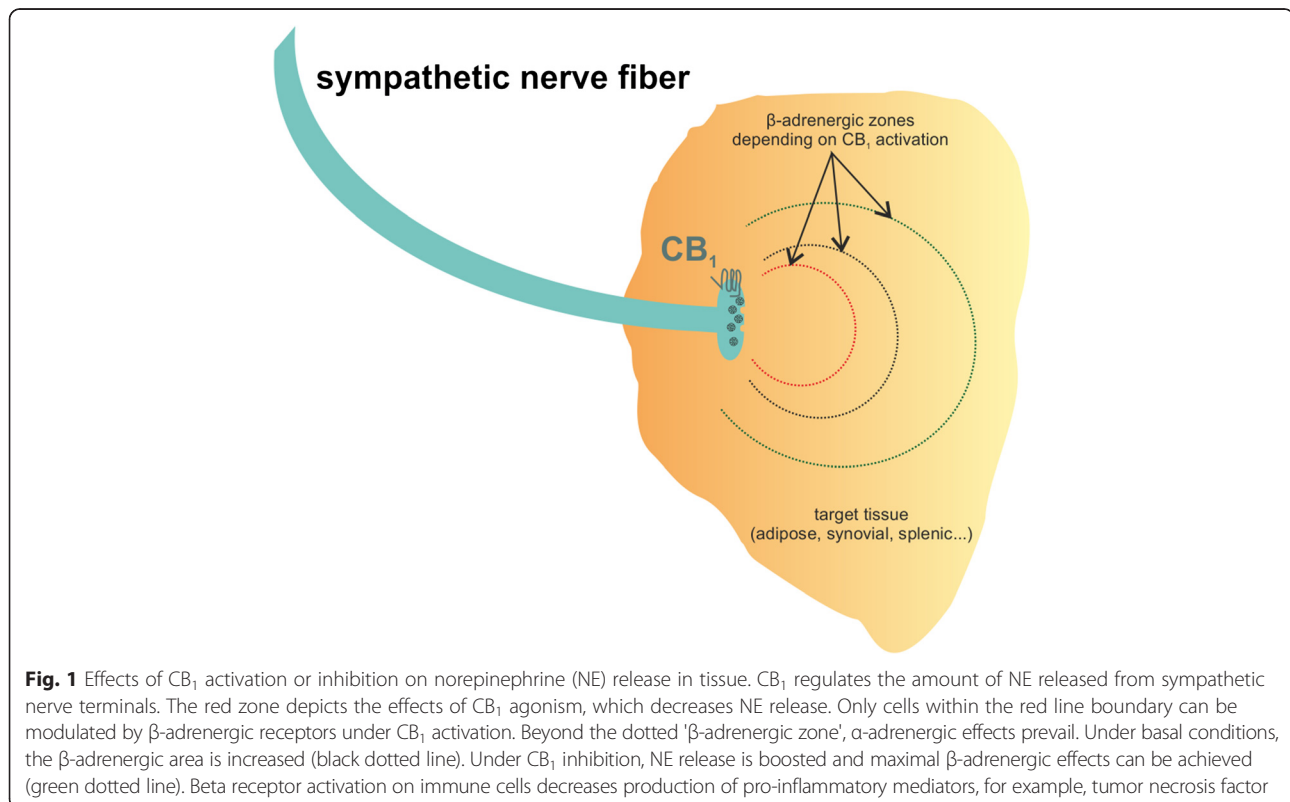
The classic function of ECs in the nervous system is the regulation of neurotransmitter release via CB₁, which is also responsible for the psychotropic effects of cannabis [20–23]. CB₁ is mainly located on presynaptic nerve terminals, and activation of this receptor reduces the release of neurotransmitter from corresponding neurons in a heteroreceptor-typical way [24]. Thus, cannabinoids can increase or decrease neuronal excitability depending on neurotransmitter and brain region affected. CB₁ receptors are also abundant on peripheral sympathetic nerve terminals, where they modulate adrenergic signaling. This influence on sympathetic nerves can alter lipolysis, cytokine production, ghrelin production, heart

rate and bone resorption [20, 25–28]. The effects of CB₁ activation or inhibition on neurotransmitter release in a given peripheral tissue are depicted in Fig. 1. In addition, CB₁ receptors are located on nociceptive nerve fibers. Here, CB₁ agonism increases the threshold for the generation of action potentials via modulation of ion channels and TRPs [29, 30].

Direct effects of CB₁ activation on immune cells have only been scarcely described. Our group but also others demonstrated an influence of cell adhesion in response to CB₁ agonism; this effect might also modulate immune function by regulating cell trafficking and tissue extravasation [31, 32].

CB₂ regulates immune cell function directly

While CB₁ functions mainly through modulation of central and peripheral neurotransmitter release, activation of CB₂ elicits direct anti-inflammatory effects in target cells [33]. This includes reduction of cytokine and matrix metalloproteinase production, modulation of adhesion and migration but also induction of apoptosis [33]. The anti-inflammatory potential of CB₂ was also confirmed in mouse models of arthritis [34, 35]. While the impact of CB₂ on immune function has already been investigated and reviewed elsewhere [33, 36], this review focuses on CB₁.



Pharmacology

Role of the ECs anandamide and 2-AG

The action of ECs is limited by rapid degradation involving FAAH, which degrades AEA and related N-acylethanolamines, and monoacylglycerol-lipase (MAGL), which degrades 2-AG [37]. In addition, several enzymes like cyclooxygenase-2, lipoxygenase or cytochrome P450 and others contribute to EC metabolism [38]. Characteristics of AEA, 2-AG, THC and the CB₁ antagonist rimonabant are given in Table 1. Inhibition of FAAH raises the levels of the N-acylethanolamines AEA, palmitoylethanolamine (PEA) and oleoylethanolamine (OEA) [39]. While AEA is responsible for maintaining basal EC signaling, 2-AG mediates strong and rapid feedback via CB₁ receptors [40]. This is also reflected by the fact that AEA is a partial agonist at CB₁, while 2-AG acts as full agonist [41]. Due to its full agonistic properties, elevation of 2-AG by inhibition of MAGL leads to functional antagonism (discussed below) of CB₁, although this might be prevented by reduced dosing [42, 43]. Furthermore, MAGL inhibition might be detrimental in some situations, since 2-AG is also degraded by cyclooxygenase-2 leading to pro-inflammatory metabolites [44]. Therefore, this review only covers the consequences of FAAH inhibition.

The conundrum of functional antagonism at CB₁ and TRPV1

Throughout this review, similar effects of CB₁ agonists and CB₁ antagonists on features of arthritic inflammation are described. This conundrum can be explained by rapid desensitization and downregulation/internalization of CB₁ upon agonist exposure [45–47]. If desensitization is disturbed due to mutations in crucial CB₁ phosphorylation sites, CB₁ agonism leads to enhanced acute effects and delayed tolerance [48]. Consequently, CB₁ signaling diminishes in response to repeated agonist exposure [49]. This feature of CB₁ explains functional antagonism: administration of exogenous cannabinoids or elevation of endogenous levels of the full CB₁ agonist 2-AG leads

to downregulation of CB₁. If levels drop low enough, production of ECs is not sufficient to activate CB₁ or CB₁ signaling pathways. This phenomenon was described with MAGL inhibitors, which increase levels of 2-AG [42]. Another possibility to achieve antagonistic effects with agonists is the use of CB₁ partial agonists like AEA, which lack full activation of CB₁ signaling pathways. These partial agonists act as antagonists when full agonists are also present [50].

TRPs, in particular TRPV1, TRPV2, TRPV3, TRPV4, TRPA1 and TRPM8, serve as ionotropic cannabinoid receptors and they also desensitize upon agonist exposure [51–55]. The EC AEA is an agonist at TRPV1 with a binding affinity similar to that of the hot pepper ingredient capsaicin, although it does not activate the receptor like capsaicin [56]. Therefore, although being an agonist itself, AEA prevents the effects of high efficacy agonists like capsaicin, thus serving as antagonist in this setting. Furthermore, AEA rapidly desensitizes TRPV1, which results in reduced calcium influx [57]. In addition, the AEA congeners and FAAH substrates PEA and OEA also desensitize TRPV1 [58, 59]. Although there are no data available regarding the desensitization of other TRPs by N-acylethanolamines, it is likely that this also occurs since there is extensive crosstalk between, for example, TRPV1 and TRPA1 via intracellular calcium [60]. Moreover, it has been demonstrated that synthetic cannabinoid ligands binding TRPA1 also desensitized target cells to the action of TRPV1 agonists [61].

FAAH inhibition does not produce central side effects and bridges TRPs and cannabinoid receptors

Central activation of CB₁ has psychotropic side effects and this problem is circumvented by the use of FAAH inhibitors [62]. In contrast to exogenous cannabinoids, AEA does not lead to tolerance at CB₁ or psychotropic effects [63]. Therapeutically, reduction of tolerance to CB₁ agonists with FAAH inhibitors can be important since this process leads to a loss of efficacy when repeatedly

Table 1 Characteristics of selected cannabinoid receptor ligands

| Ligand | Target receptors | K _i at CB ₁ in nM | K _i at CB ₂ in nM | E _{max} /IC ₅₀ at TRPV1 (nM) | Route of degradation |
|------------|---|---|---|--|--------------------------------------|
| Anandamide | CB ₁ , CB ₂ , GPR55, TRPV1, TRPA1, TRPM8 (antagonist) | 239.2 ± 61.77 [158] | 439.5 ± 95.89 [158] | 458 (E _{max}) [159] | FAAH, FAAH-2, NAAA, COX-2, LOX [160] |
| 2-AG | CB ₁ , CB ₂ , TRPV1, GABAA | 3423.6 ± 3288.24 [158] | 1193.8 ± 327.71 [158] | 750 ± 40 (IC ₅₀) [161] | MAGL, COX-2, LOX, ABHD6/12 [160,162] |
| Delta9-THC | CB ₁ , CB ₂ , GPR18 | 25.1 ± 5.54 [158] | 35.2 ± 5.86 [158] | NA | CYP2C [163] |
| Rimonabant | CB ₁ , MOR | 1.98 ± 0.36 [164] | NA | NA | CYP3A [165] |

Anandamide, 2-arachidonylglycerol (2-AG) and tetrahydrocannabinol (THC) are CB₁/CB₂ agonists, rimonabant is a CB₁/MOR antagonist/inverse agonist. Anandamide and THC are partial CB₁/CB₂ agonists, 2-AG is a full agonist at both receptors. The main degrading enzyme for each compound is highlighted in bold. *ABHD*, α/β-hydrolase domain; *CB₁/CB₂*, cannabinoid receptor I/II; *COX-2*, cyclooxygenase-2; *CYP*, cytochrome P450; *Delta9 THC*, delta9 tetrahydrocannabinol; *E_{max}*, maximal functional response; *FAAH*, fatty acid amide hydrolase; *IC₅₀*, half maximal inhibitory concentration; *K_i*, dissociation constant; *LOX*, lipoxygenase; *MAGL*, monoacylglycerol lipase; *MOR*, μ opioid receptor; *NA*, not applicable; *NAAA*, N-acylethanolamine-hydrolyzing acid amidase; *TRPA1*, transient receptor potential ankyrin I; *TRPM8*, transient receptor potential melastatin 8; *TRPV1*, transient receptor potential vanilloid I

administered [63]. In addition, elevation of OEA and PEA also provide anti-inflammatory, neuroprotective effects and they enhance neurogenesis mostly via peroxisome-proliferator activated receptors [64–66]. FAAH inhibition has already been demonstrated to be effective in collagen-induced arthritis in mice, although this was attributed to CB₂ activation [34]. Furthermore, FAAH inhibition not only combines anti-inflammatory effects of several N-acyl ethanolamines but also targets additional receptors such as TRPV1 and peroxisome proliferator activated receptors [65, 67–69]. One important receptor for AEA and its congeners OEA and PEA is the TRPV1 cation channel, although other TRPs are similarly activated by AEA [69–71].

Besides CB₁ and CB₂, ECs as well as synthetic and phytocannabinoids bind to members of the TRP family [54, 61, 72–74]. Several of these non-selective cation channels integrate external and endogenous stimuli and are sensitized and activated during inflammation [19, 75]. Pharmacological elevation of AEA in the rat leads to activation but also desensitization of TRPV1, resulting in increased pain thresholds [69]. In contrast to CB₁ activation, TRP activation increases cell excitability leading to increased release of neurotransmitters [76–78]. When co-expressed, CB₁ agonism decreases TRPV1 channel activity by dephosphorylation, which increases the threshold for agonists [78]. Although mainly located on sensory A δ and C-fibers, TRPs are also expressed on peripheral cells such as synoviocytes, and activation results in increased expression of inflammatory mediators [75, 79, 80]. The best described example of subsequent TRPV1 and CB₁ activation is the regulation of blood pressure, where only the CB₁/TRPV1 agonist AEA elicited a triphasic response involving both receptors [81]. First, AEA activates TRPV1 causing hypotension and bradycardia followed by a pressor phase with increased heart rate. In the final phase, prolonged hypotension by AEA is observed and this effect was inhibited by CB₁ antagonism. The sequential activation of TRPV1 and CB₁ in the context of blood pressure regulation has been reviewed elsewhere [81].

Clinical relevance

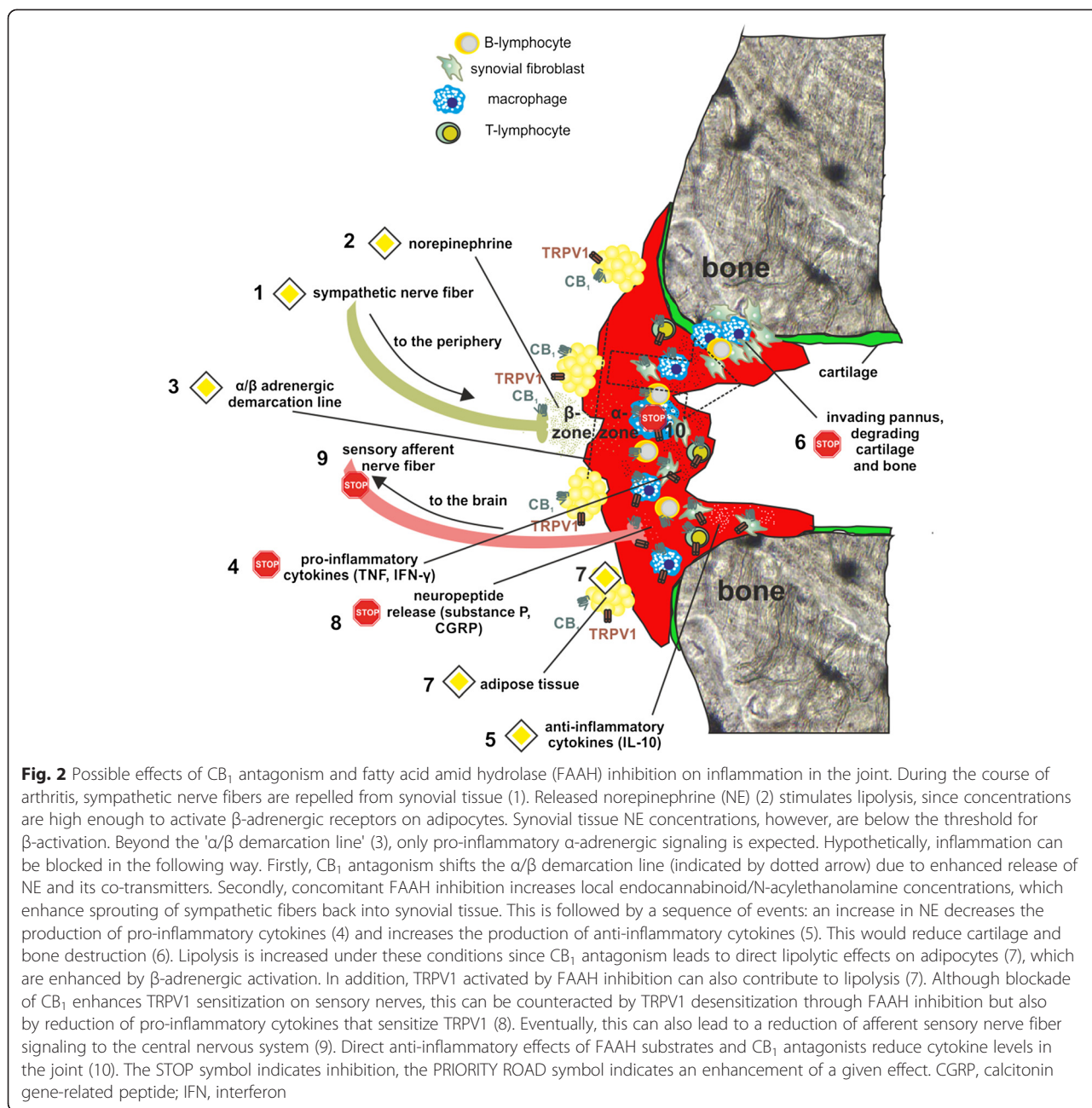
The sympathetic nervous system supports chronic inflammation in arthritis - links to endocannabinoids

Sympathectomy in arthritic patients has already been performed in the 1920s and follow-up studies showed reduced joint swelling and pain in sympathectomized patients [82]. The neuroinflammatory component of arthritis has been revealed in studies by Levine and colleagues [83, 84]. In the mouse model of collagen-induced arthritis it was shown that chemical sympathectomy before or during the time of immunization results in less severe disease [85]. Late sympathectomy, however, results in exacerbation of experimental arthritis, which might be due to deletion of tyrosine hydroxylase-positive catecholamine-

producing cells that appear in synovial tissue during the course of the disease [86]. The beneficial effects of tyrosine hydroxylase-positive cells on the development of collagen-induced arthritis was demonstrated by our group. *In vitro*, tyrosine hydroxylase controls cytokine production in mixed synovial cells, whereas *in vivo* introduction of these cells into arthritic mice reduced arthritic score [87]. During arthritic inflammation in mice and humans, production of nerve repulsion factors by macrophages leads to the retraction of sympathetic but not sensory fibers from synovial tissue [88]. As a result, synovial concentration of norepinephrine falls under the threshold for anti-inflammatory β 2 receptor activation and this favors pro-inflammatory effects via α -adrenergic signaling [89, 90]. However, sympathetic signaling is increased in adipose tissue surrounding the synovium, which is responsible for generating energy-rich substrates to support inflammation [91]. These changes in sympathetic activity during the course of arthritis might be limited or even reversed by altering either EC production or CB₁ function, since this receptor controls norepinephrine release. Reduction of EC production by blocking appropriate synthesizing enzymes leads to a functional loss of CB₁ since low levels of ECs can no longer activate the receptor. This was already demonstrated in a mouse model of constipation, where inhibition of diacylglycerol lipase α lowered levels of the CB₁ agonist 2-AG with concomitant increases in gut motility [92]. The same effect is achieved by antagonizing CB₁ directly [93]. The loss of sympathetic nerves, altered adrenergic signaling and the possible influence of ECs in the joint is visualized in Fig. 2. In parallel with the disappearance of sympathetic nerve fibers in the joint, hypothalamic norepinephrine, interleukin (IL)-6 and IL-1 β increase during the induction phase of experimental arthritis [94] (Fig. 3). In addition, these changes in cytokine levels and disruption of adrenergic signaling are not accompanied by an adequate response of the hypothalamus-pituitary-adrenal (HPA) axis, resulting in low cortisol levels in relation to inflammation in humans and rodents [94]. A more detailed description of the influence of the sympathetic nervous system on inflammation has recently been published by our group [95].

Modulation of adrenergic signaling via CB₁ might be beneficial in arthritis

In adjuvant arthritis, immune cells respond to adrenergic β 2 receptor stimulation with decreased production of tumor necrosis factor (TNF), an increase in anti-inflammatory IL-10, and a shift to a T-helper type 2 and T-regulatory immune response [96]. Antagonism of CB₁ at splenic sympathetic terminals provides strong anti-inflammatory effects and ameliorates collagen-induced arthritis in mice via reduction of TNF levels, which was inhibited by β 2 adrenergic antagonism [26] (Fig. 4).



Furthermore, β 2 adrenergic activation on murine B-lymphocytes increases production of the anti-inflammatory cytokine IL-10, which inhibits inflammation [97]. The time window for anti-arthritic β 2 adrenergic effects in mice is crucial since early activation (during the induction phase of experimental arthritis) of sympathetic signaling in the spleen increases interferon (IFN)- γ production [98]. Sympathetic innervation of the spleen is reduced during the course of experimental arthritis, comparable to the situation in synovial tissue [99]. This has profound effects on local adrenergic signaling since low concentrations of norepinephrine favor pro-inflammatory α -adrenergic receptor

activation [100, 101] (Fig. 4). Although the beneficial outcome of CB₁ receptor antagonism in collagen-induced arthritis in mice was attributed to β 2-receptor activation on splenocytes, several other mechanisms might contribute to the therapeutic effects. CB₁ antagonism at sympathetic terminals surrounding the synovium might have different outcomes depending on the magnitude of recovery of norepinephrine levels in the joint. If β 2 signaling is restored in synovial tissue, local concentrations of IFN- γ and TNF might decline, leading to an overall decrease in joint destruction, synovial inflammation and pain [102, 103] (Fig. 2). On the other hand, since we demonstrated an

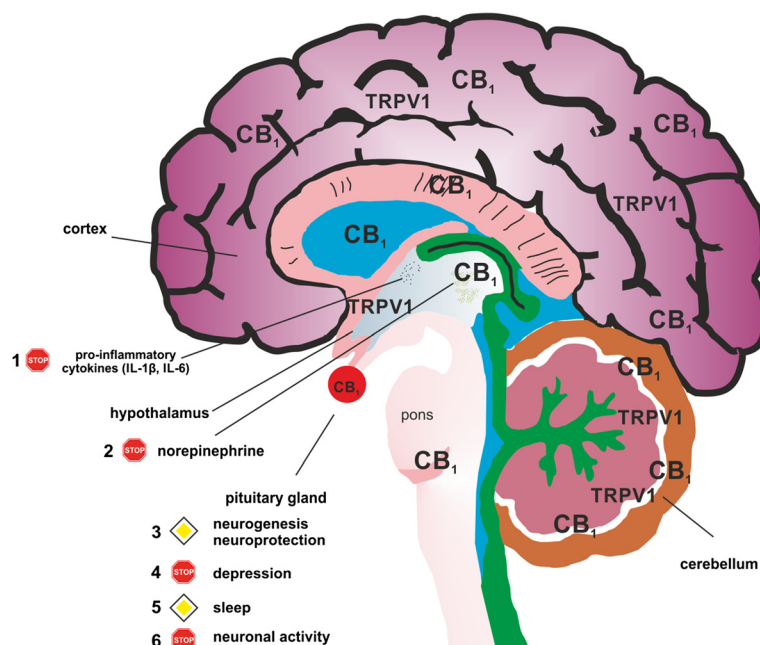


Fig. 3 Possible effects of fatty acid amid hydrolase (FAAH) inhibition on neuroinflammation. CB₁ and TRPV1 are expressed throughout the brain by several cell types, including microglia. In addition, FAAH-degradable N-acylethanolamines activate several other anti-inflammatory pathways supporting the role of CB₁. Since no data are available regarding the effects of FAAH on sympathetic activity or microglia, the following sequence is hypothetical in nature. Upon activation, microglia produce pro-inflammatory cytokines and CB₁ activation opposes this (1). Since CB₁ controls neurotransmitter release, hypothalamic norepinephrine is decreased by FAAH inhibition, restoring brain-immune system-joint communication (2). Damaged neuronal tissue generated by the pro-inflammatory milieu is regenerated by CB₁ activation (3). FAAH inhibition elevates mood and depressive symptoms in patients disappear due to decreased brain cytokines levels (4). Rheumatoid arthritis patients often suffer from bad sleep quality, and this is surpassed by FAAH inhibition (5). In general, CB₁ activation decreases neuronal excitability, and this supports the general anti-inflammatory effect on microglia, which are activated by glutamate (6). The STOP symbol indicates inhibition, the PRIORITY ROAD symbol indicates an enhancement of a given effect

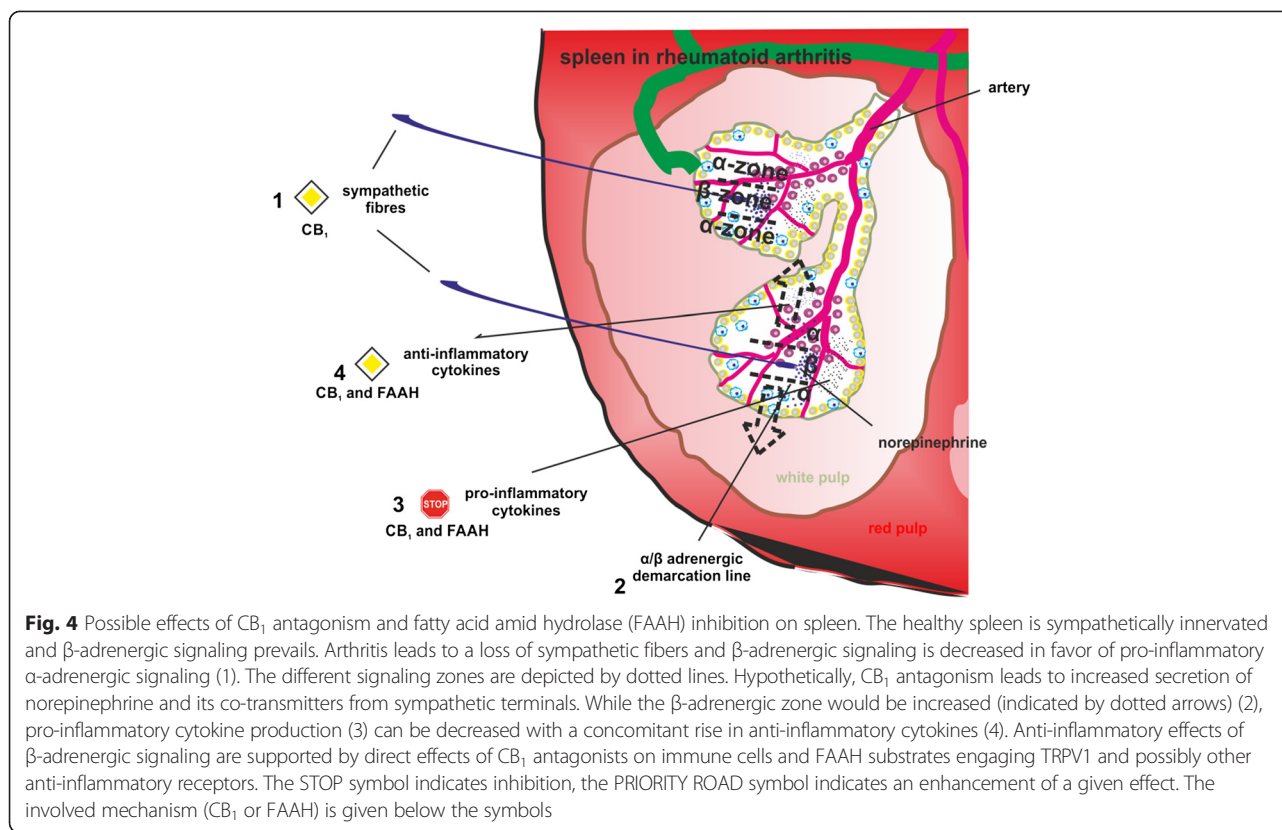
increase of sympathetic fibers in human synovial adipose tissue, increased norepinephrine release might further increase lipolysis and thereby fuel inflammation [91]. Thus, it is imperative to maintain norepinephrine levels over a certain ' β 2 activation threshold' in the synovium, which might only be achieved with continuous high doses of CB₁ antagonists. Consequences of enhanced β 2 signaling by CB₁ antagonism are depicted in Fig. 2.

Although the above mentioned stimulating effects of CB₁ antagonism on adrenergic signaling are evident, CB₁ agonists might also prove useful in modulating arthritis. As mentioned earlier, sympathectomy in the early phase ameliorates experimental arthritis in mice [85]. This indicates a pro-inflammatory influence of adrenergic signaling at the beginning of the disease, which might be counteracted by CB₁ agonists decreasing norepinephrine levels [20]. Arthritis is accompanied by a loss of sympathetic nerve fibers from sites of inflammation and this might also be counteracted by CB₁ activation, since neurogenesis is disturbed in CB₁ knock-out mice, although we do not know whether this also applies for sympathetic nerve fibers [104].

The development of comorbidities such as bone resorption, depression and water retention/volume expansion in RA is partly driven by changes in sympathetic activity [19, 105]. Osteoporosis is a major contributor to RA-associated complications and osteoclasts and osteoblasts respond to cannabinoid receptor activation [106, 107]. Activation of CB₁ results in enhanced osteoblast differentiation, which leads to reduced osteoporosis. Blockade of CB₁ disturbs osteoclast function and increases bone mass in the young, but leads to osteoporosis later on due to decreased bone formation [108].

One major disability associated with RA is the development of depression, which affects around 17 % of patients and is associated with poorer disease outcome [109]. Depression and CB₁ are connected since side effects of rimonabant, a first generation CB₁ inverse agonist/antagonist, include depression and anxiety while CB₁ agonism has anxiolytic-like and antidepressant-like activities [110, 111]. The effects of CB₁ agonism by FAAH inhibition in the brain are depicted in Fig. 3.

Overactivity of the sympathetic nervous system in RA also leads to water retention via activation of the renin-



aldosterone-angiotensin system [19]. Cannabinoids not only induce diuretic effects but also decrease aldosterone secretion from the adrenal glands by activation of CB_1 [112, 113].

CB_1 antagonism activates the HPA axis and reverses insulin resistance

Although modulation of immune cell function via β_2 -adrenergic receptors is important, CB_1 antagonism also supports beneficial systemic changes. One of the hallmarks of RA is an inadequate cortisol secretion in relation to inflammation [114]. Antagonism at CB_1 might counteract this phenomenon, since CB_1 knock-out mice had higher levels of adrenocorticotrophic hormone and corticosterone under basal but also under stressed conditions [115]. ECs control glucocorticoid feedback and, therefore, CB_1 antagonism increases circulating adrenocorticotrophic hormone levels [116]. Interestingly, high doses of a CB_1 agonist also increase the activity of the HPA axis, although this is due to alteration of serotonergic and adrenergic transmission [117]. The same outcome using CB_1 antagonism or agonism on HPA axis activation might also depend on the concentration of CB_1 agonists and whether central or peripheral CB_1 receptors are targeted. Peripheral agonism at CB_1 leads to subsequent activation of α and β adrenoreceptors, which are linked to the antinociceptive effects of CB_1 in

a rat pain model [118]. Increases in adrenergic signaling by CB_1 agonists might be due to decreased inhibitory gamma-aminobutyric acid (GABA) signaling since release of this neurotransmitter is also controlled by CB_1 [22]. Thus, enhanced GABA signaling reduces sympathetic activity and vice versa [119]. Central activation of CB_1 mediates the rapid effect of glucocorticoid negative feedback and this might explain the necessity for high peripheral doses of the CB_1 antagonist rimonabant to increase cortisol levels [120, 121].

A major problem during the course of RA is the development of insulin resistance with systemic metabolic changes [122, 123]. Insulin resistance is a direct consequence of enhanced pro-inflammatory cytokine signaling and TNF, IL-6, IL-1 β as well as other cytokines are responsible for these changes [124]. From 2006 to 2008 the CB_1 antagonist rimonabant was marketed for use against obesity but was withdrawn due to central side effects [125]. However, the drug proved to be effective at decreasing important parameters associated with metabolic syndrome. Rimonabant reduces leptin expression, decreases atherosclerosis, and reverses insulin resistance in rodents and humans [126, 127]. In this respect, CB_1 antagonism might also be beneficial in reversing metabolic changes in RA. Insulin resistance is induced by the immune system to divert energy to active immune cells, which are not dependent on insulin for glucose utilization

[105]. Therefore, CB₁ antagonism might normalize energy distribution throughout the body and this might deprive the activated immune system of nutrients important for the perpetuation of inflammation. Interestingly, CB₁ activation by the phytocannabinoid δ 9-tetrahydrocannabinol (THC) corrects hyperlipidemia and hyperglycemia [128]. This effect might be relevant when using CB₁ partial agonists like THC that act as antagonists when full agonists like the EC 2-AG are present. In mice, THC antagonized the effects of the synthetic CB₁ agonist AM2389 on hypothermia, although it elicited hypothermic effects by itself [129]. Furthermore, repeated administration of cannabinoids leads to desensitization and downregulation of CB₁, resulting in functional antagonism [42, 48].

Systemic leptin levels are decreased by CB₁ inhibition, and this adipokine is associated with higher IL-6 production and it also initiates production of TH1 cytokines [130, 131]. In addition, cardiovascular events are a major risk in RA and CB₁ antagonists might be effective in decreasing vascular inflammation [132, 133].

Direct anti-inflammatory effects of CB₁ on immune cells

Although most changes associated with CB₁ antagonism are mediated via the sympathetic nervous system, direct effects on the immune system are also described. In macrophages from CB₁ knock-out mice, TLR4 expression and concomitant pro-inflammatory cytokine production were reduced [134]. Anti-inflammatory effects of CB₁ inhibition were also demonstrated in THP-1 macrophages, where rimonabant decreased TNF and increased IL-10 production [135]. Furthermore, in a mouse model of sponge-induced angiogenesis, CB₁ antagonism reduced leukocyte infiltration and chemokine/cytokine production [136].

CB₁ agonism also has anti-inflammatory effects on immune cells - for example, decreased activation of T lymphocytes by downregulating IL-2 [137]. However, direct effects of CB₁ agonists are most prominent when injected into the brain, where CB₁ activation reduces the severity of intestinal inflammation and decreases the activity of microglial cells via reduction of pro-inflammatory cytokines in mice [138, 139]. Therefore, CB₁ activation might alleviate arthritis through central nervous pathways, since neuroinflammation and concomitant increases in brain cytokine levels contribute to the disease [94, 140].

Central effects of CB₁ ligands limit their therapeutic use

Although therapeutically active when administered intrathecally, the use of CB₁ agonists or antagonists is limited due to their central adverse events. While CB₁ antagonists/inverse agonists like rimonabant induce depression and anxiety in some patients, CB₁ agonists like THC have psychotropic properties [110, 141]. This might derive from reduction of glutamatergic neurotransmission in response

to CB₁ activation leading to effects similar to NMDA antagonism [142]. This problem might be circumvented by using peripherally restricted CB₁ ligands, which have been generated as second generation cannabinoid therapeutics with proven effects [143, 144]. Furthermore, neutral antagonists with limited brain penetration and which lack the adverse effects of the inverse agonist rimonabant have been developed [145]. Neutral antagonists do not influence the constitutive activity of CB₁ and therefore do not mediate some of the adverse effects observed with rimonabant therapy [146]. In contrast to neutral antagonists, inverse agonists like rimonabant not only block CB₁ but also stabilize the receptor in an inactive conformation. This diminishes basal signaling and leads to a reciprocal receptor response. In the case of CB₁, cAMP is increased by inverse but not by neutral antagonists [146, 147].

Crosstalk between CB₁ and TRPV1 modulates pain and inflammation in arthritis

The importance of TRPV1 in arthritis is emphasized in knock-out animals that show an attenuated disease [148, 149]. In TRPV1^{-/-} animals, pain thresholds were increased with a concomitant reduction of joint inflammation [149]. The same protective effect was achieved by oral administration of the TRPV1 agonist SA13353, which reduced TNF production and provided antiarthritic effects in the rat [150]. Interestingly, this effect was mediated by TRPV1 located on sensory neurons, emphasizing the neuronal component of arthritis [150]. This might disrupt a positive feedback loop, since TNF and other pro-inflammatory cytokines sensitize TRPV1 and enhance its activity [102]. The paradoxical finding that TRPV1 agonists also act in an anti-inflammatory fashion is explained by rapid desensitization of TRPV1 in response to agonist treatment, which depends on the agonist used [151]. Findings in synovial fibroblasts support this notion, where the TRPV1 agonist capsaicin increases IL-6 production, while AEA, a low efficacy TRPV1 agonist, decreased IL-6 levels under TNF stimulation (T Lowin, unpublished data) [80].

Since some peripheral effects of TRPV1 are attributed to receptors located on sensory nerve terminals which co-express CB₁, crosstalk between both receptors might define the outcome of inflammation [152]. This can be important in RA, since elevated synovial levels of nerve growth factor sensitize TRPV1 to inflammatory stimuli and CB₁ agonism counteracts this response [153, 154]. In this respect, FAAH inhibition might be superior to selective CB₁ agonists since AEA or its metabolites not only activate CB₁ but also desensitize TRPV1, leading to analgesia [69]. Neuronal TRPV1 increases neurotransmitter and pro-inflammatory neuropeptide release via elevation of intracellular calcium levels and the same mechanism often induces the secretion of cytokines

from immune cells [155–157]. Inhibition of TRPV1 function by concomitant CB₁ activation and AEA-induced desensitization (FAAH inhibition) might be a promising strategy to reduce RA disease activity and pain.

Conclusion: is there a perfect cannabinoid-based therapy for the treatment of RA?

The question arises how to modulate the EC system for the treatment of RA. The best treatment option might be a combination of a peripherally restricted CB₁ antagonist and a FAAH inhibitor raising systemic levels of N-acylethanolamines. CB₁ antagonism has already been shown to result in anti-arthritic effects in mice and this treatment might also increase adrenergic signaling in RA, thereby reducing TNF and IFN- γ and decreasing joint inflammation and cartilage destruction. Potential effects of CB₁ antagonism (also of FAAH inhibition) in arthritic synovium and spleen are shown in Figs. 1 and 3, respectively.

Furthermore, CB₁ antagonists might reverse metabolic alterations associated with RA: for example, insulin resistance, enhanced leptin expression, depression/fatigue or atherosclerosis. FAAH inhibition on the other hand can counteract the neuroinflammatory component of RA by activating neuronal CB₁ and TRPV1 (Fig. 3). Furthermore, the FAAH substrates OEA and PEA can support anti-inflammatory and neurogenic effects of central CB₁ activation via peroxisome-proliferator activated receptors. In addition, CB₁ activation in the brain lowers sympathetic activity, which can decrease disease-related problems like hypertension. In addition, increases in brain AEA can have antidepressant effects and since many RA patients suffer from mood disorders, FAAH inhibition might help to counteract this central nervous system problem.

In the periphery, FAAH inhibition leads to analgesic and anti-inflammatory effects via desensitization of TRPV1. Moreover, FAAH inhibition has been shown to have high efficacy in arthritic mice through activation of CB₂, which might also be beneficial in patients by downregulating cytokine production. In summary, therapeutic intervention in RA with a peripherally restricted CB₁ antagonist and a FAAH inhibitor might offer a promising strategy to ameliorate RA.

Abbreviations

2-AG: 2-arachidonylglycerol; AEA: Arachidonylethanolamine; EC: Endocannabinoid; FAAH: Fatty acid amid hydrolase; GABA: Gamma-aminobutyric acid; HPA: Hypothalamus-pituitary-adrenal; IFN: Interferon; IL: Interleukin; MAGL: Monoacylglycerol-lipase; OEA: Oleoylethanolamine; PEA: Palmitoylethanolamine; RA: Rheumatoid arthritis; THC: Tetrahydrocannabinol; TNF: Tumor necrosis factor; TRP: Transient receptor potential channel.

Competing interests

The authors declare that they have no competing interests.

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