From Cannabis to Endocannabinoids in Multiple Sclerosis: A Paradigm of Central Nervous System Autoimmune Diseases

Anna Maria Malfitano[1], Giuseppe Matarese[2] and Maurizio Bifulco[*,1]

[1]*Dipartimento di Scienze Farmaceutiche, Universita' di Salerno, Fisciano (Sa), Italy*

[2]*Gruppo di ImmunoEndocrinologia, Istituto di Endocrinologia e Oncologia Sperimentale del CNR e Dipartimento di Biologia e Patologia Cellulare e Molecolare, Universita di Napoli Federico II, Napoli, Italy*

Abstract: An Increasing body of evidence suggests that cannabinoids have beneficial effects on the symptoms of multiple sclerosis, including spasticity and pain. Endogenous molecules with cannabinoid-like activity, such as the "endocannabinoids", have been shown to mimic the anti-inflammatory properties of cannabinoids through the cannabinoid receptors. Several studies suggest that cannabinoids and endocannabinoids may have a key role in the pathogenesis and therapy of multiple sclerosis. Indeed, they can down regulate the production of pathogenic T helper 1-associated cytokines enhancing the production of T helper 2-associated protective cytokines. A shift towards T helper 2 has been associated with therapeutic benefit in multiple sclerosis. In addition, cannabinoids exert a neuromodulatory effect on neurotransmitters and hormones involved in the neurodegenerative phase of the disease. *In vivo* studies using mice with experimental allergic encephalomyelitis, an animal model of multiple sclerosis, suggest that the increase of the circulating levels of endocannabinoids might have a therapeutic effect, and that agonists of endocannabinoids with low psychoactive effects could open new strategies for the treatment of multiple sclerosis.

Keywords: MS, CNS, endocannabinoids, T cells, cytokines.

Multiple sclerosis (MS) is one of the most common chronic and disabling disorders of the central nervous system (CNS) caused by demyelination (loss of insulating sheath) of nerve fibres. The disease usually begins in young adulthood and affects women more frequently than men (2:1). Common symptoms include fatigue, balance problems, muscle weakness, incontinence, muscle spasm, pain and tremor. Clinical studies indicate that MS is characterised by at least two distinct phases, one that is dominated by acute relapses and one by steady progression. Both genetic and environmental factors seem to contribute synergistically to the manifestation and progression of the disease. MS usually starts with a relapsing–remitting course (RR-MS); over time, the number of relapses decreases, and most patients develop progressive neurological deficits that occur independently of relapses (the so-called secondary progressive phase). In few cases, MS begins with a primary progressive course (PP-MS) without acute relapses. In general, the progression rate in RR-MS is comparable to that of PP-MS as soon as the patients enter the secondary progressive phase [1]. CNS lesions, frequently detected in RR-MS phase [2], are usually located in areas of white matter, and are often characterised by a disturbance of the blood–brain barrier, local oedema and demyelination, features that are compatible with an inflammatory process, while in PP-MS, such inflammatory activity is much less conspicuous [2]. Global brain atrophy, however, is more dominant in the progressive stage and seems to correlate with disability [3, 4]. These findings indicate that early in the disease, ongoing inflammatory activity is present in most patients and is responsible for the relapsing–remitting course, whereas a distinct process might be operative in the progressive phase of the disease, when inflammatory activity diminishes despite faster evolution of disability.

Histological hallmarks of active MS include infiltrations of T cells, macrophages, and B cells, degradation of myelin, and, to a lesser extent, axons, and reactive changes of astrocytes and microglia [5]. Autoimmunity is thought to drive the development of inflammatory lesions that induce the primary demyelination, which results in the inhibition of normal neurotransmission [6]. Current treatment of MS is based on anti-inflammatory, immunosuppressive and immunomodulatory drugs, but usually the therapy is partially effective and with risks of side effects that patients are often unable to tolerate. This has led some multiple sclerosis patients to self-medicate with cannabis, which is suggested by anecdotal evidence to be beneficial in controlling symptoms such as spasticity, pain, tremor and bladder dysfunction.

Cannabis contains a series of compounds, but it has been found that the major psychoactive ingredient is Δ^9-tetrahydrocannabinol (THC) [7]. Two selective cannabinoid receptor subtypes have been identified so far [8, 9], CB1 and CB2, that are expressed in nervous and peripheral cells. THC mediates the majority of its activities through stimulation of cannabinoid receptors CB1, which are expressed throughout the CNS [10, 11]. Following the discovery of the receptors, fatty acid endogenous ligands, such as anandamide and 2-arachidonoyl glycerol (2-AG), have been discovered in mammalian and human nervous tissues [12-14], and a degradation system including a re-uptake mechanism and hydrolytic enzymes has been identified [12, 13, 15, 16]. The

*Address correspondence to the authors at the Dipartimento di Scienze Farmaceutiche, Universita' di Salerno, *Via* Ponte don Melillo 84084 Fisciano (Salerno), Italy; Tel: 39-089/962516; Fax: 39-089/962828; E-mail: maubiful@unina.it

1568-007X/05 $50.00+.00

cannabinoid system functions to regulate synaptic neurotransmission [17, 18] and tonically controls clinical signs such as spasticity and tremor that develop in murine models of MS [19, 20]. This provides objective evidence to support the claims of multiple sclerosis patients that cannabis may have a benefit in symptom management [21], a claim further supported by some recent clinical trials of medical cannabis extracts [22-24]. There is *in vitro* evidence showing that cannabinoids can also regulate glutamate release, oxidant free radicals and calcium influxes [25, 26, 17, 11], which, in excess, can cause neuronal death in neuroinflammatory disease [27-29]. The lack of specificity of all available cannabinoid reagents [11] suggests the possibility of additional CB-like receptors [30-32]. Gene-deleted transgenic mice [33, 34] provide powerful tools to definitively investigate the potential role of the cannabinoid system in neuroprotection.

Recent studies have suggested that cannabinoid-based treatments may be beneficial in a wide number of diseases. The pharmacological activity of anandamide and 2-AG has been thoroughly examined and shown to be similar to that of some psychotropic plant cannabinoids, namely THC [35-37]. In particular, they have been found to exert a neuromodulatory effect [38] on the synthesis, release and action of neurotransmitters. Some of these neurotransmitters, e.g. dopamine, γ-aminobutyric acid and glutamate, have been recently implicated in the genesis of experimental autoimmune encephalomyelitis (EAE) [39, 40, 27], an animal model of inflammatory disease of the CNS myelin. EAE is induced by immunization of susceptible animals with myelin antigens and freund's adjuvant [41] and reproduces the features of MS in rodents. Several research groups suggest that both cannabinoids and ECs modulate the levels and action of several of these mediators. Cannabinoids and ECs may play a pivotal role in the pathogenesis of MS, probably through the cannabinoid receptors CB1 and CB2. The target of the inflammatory response in MS is myelin and the myelin producing cells, such as the oligodendrocytes. Infiltration of activated immune cells and recruitment of endogenous glial cells lead to the destruction of myelin and oligodendrocytes through the release of pro-inflammatory cytokines (Fig. **1**) by the former cells and subsequent disruption of the blood-brain barrier permeability [42]. In fact, recent studies suggested that cytokines play a crucial role in the clinical course of EAE and MS. With the introduction of T-cell cloning technology, it was possible to show that in EAE, T helper (Th) 1 cytokines such as interferon-γ (IFN-γ), tumour necrosis factor-α (TNF-α), and interleukin 2 (IL-2), were more likely to transfer the disease than other myelin-specific T cells [43]. By contrast, T cells that secreted IL-4, IL-5, IL-10 and IL-13 seem to protect animals from the disease [44]. Further support that the disease results from a Th1-driven immune process was provided by a clinical trial showing that administration of the Th1 cytokine IFN-γ exacerbates MS [45]. On the basis of these findings, it is widely accepted that EAE, a prototypic autoimmune disorder, is caused by a Th1 cell response to myelin antigens.

CANNABINOIDS AND IMMUNE CELLS

The effect of cannabimimetic agents on the function of immune cells such as T and B lymphocytes, natural killer cells and macrophages has been extensively studied over the past several decades using human and animal paradigms, involving whole animal models as well as tissue culture systems. These drugs have subtle complex effects on immune cell function and some of the drug activity is mediated by cannabinoid receptors expressed on the various immune cell subtypes. It is known that CB1 mRNA levels varied among cell subpopulations. In human peripheral blood mononuclear cells (PBMC), the mRNA was reported to be most abundant in B cells, and the rank order was B cells > NK cells > polymorphonuclear neutrophils (PMNs) > CD8 cells > monocytes > CD4 cells [46]. CB1 mRNA levels also varied in mouse immune subpopulation, and consistent with human cells, splenic B cells expressed more mRNA than macrophages or T cells [47-49]. CB2 mRNA expression in PBMC was in the rank order of B cells > natural killer (NK) cells > monocytes > PMNs > T cells. Many studies suggested that CB2 mRNA was expressed primarily in immune cells but not in brain and that it was most abundant in B cells [48, 50-52]. The high expression of CB2 receptors in B cells and natural killer cells suggests that this subtype contributes to the potential immunosuppressant and anti-inflammatory effects of cannabinoids [53]. It was shown that treatment of mice and rats with THC or treatment of immune cells cultures from humans and rodents has, for the most part, a suppressive effect on T and B cells, NK cells, and macrophages. Various functions from cytotoxic T cells killing to antibody production by B cells, and to phagocytosis and killing by macrophages have been studied. Most functions were suppressed by drug treatment, and the effective drug concentrations *in vitro* were in the μM range. As this concentration range was higher than the reported receptor affinities, it was concluded by many authors that non-specific effects of these lipophilic drugs on cell membranes primarily mediated the effects of cannabinoids on immune cell function. In murine studies, cannabinoids were reported to down regulate the production of T helper 1 (Th1)-associated cytokines, and increase the production of T helper 2 (Th2)-associated cytokines [54-58]. A polarisation of T cell response towards a Th2 phenotype has been associated with therapeutic benefit in MS, while a shift towards Th1 has been associated with disease progression [59]. It was shown that THC injection in mice infected with *Legionella pneumophila*, suppressed Th1 immunity by inhibiting the production of IFN-γ and IL-12 as well as the expression of IL-12 receptors, and in addition, drug treatment increased the expression of Th2 promoting cytokine, IL-4 [55]. A similar shift to Th2 cytokines was demonstrated in response to THC in activated peripheral blood T cell cultures [61]. In drug treated cultures, proliferation was inhibited along with Th1 cytokines; Th2 cytokines were increased, and the CB2 antagonist inhibited the effects [61]. There is ample evidence that cannabinoids can modulate the development of Th, thus it is possible that CB1 and CB2 may be differentially expressed on different subpopulations of Th cells. This selective expression could lead to an increase in Th2 development and a decrease in the development of Th1 cells, resulting in decreased cell-mediate immunity and increased antibody immunity. Further research on the function and distribution of cannabinoid receptors as well as the production of endocannabinoids by

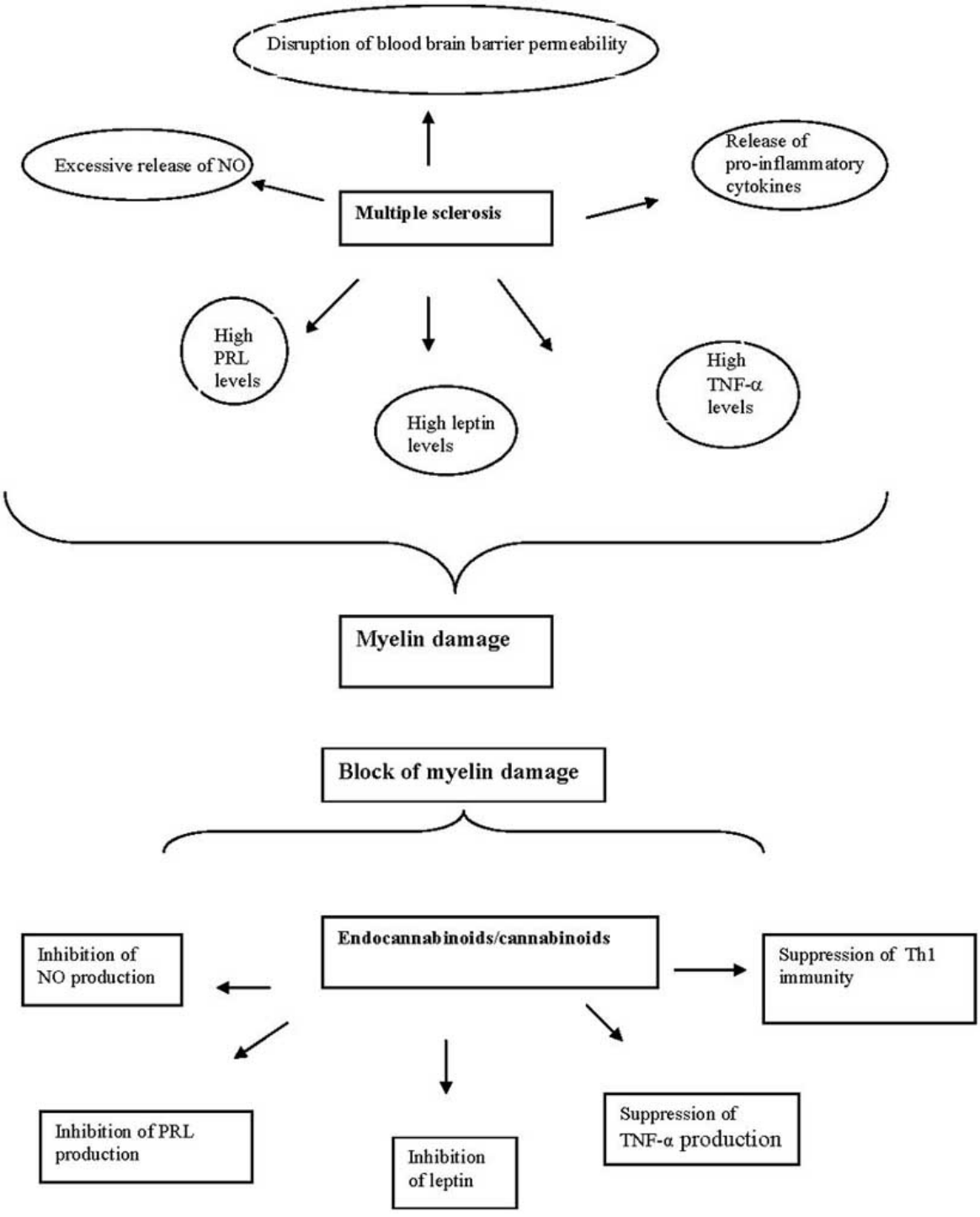

Fig. (1). Factors involved in myelin damage and effexts of ECs and cannabinoids.

different immune cells will provide greater insight into these mechanisms.

CYTOKINES, INFLAMMATORY MEDIATORS AND CANNABINOIDS

IL-6 is an important mediator of inflammatory and immune responses in the periphery, and induces growth and differentiation of cells in the immune and haematopoietic systems. IL-6 is also produced within the CNS and is involved in cell-cell signalling, coordination of neuroimmune responses, protection of neurons and neuronal differentiation, growth and survival. IL-6 plays an important role in both physiological and path physiological processes in the CNS. IL-6 is one of the several cytokines upregulated

in blood mononuclear cells and, particularly, in the cerebrospinal fluid (CSF) of patients with MS [61]. The EC anandamide enhances IL-6 production by astrocytes in a concentration-dependent manner in an MS model, the Theiler's Murine Encephalomyelitis virus (TMEV) infection. SR141716A, a potent and selective antagonist of the CB1 cannabinoid receptor, blocks the enhancing effects on IL-6 release, thus suggesting a cannabinoid receptor-mediated pathway [62]. Very recent reports strongly suggest a TNF-α pathogenic role in demyelination in human MS [63]. Cannabinoids inhibit LPS-induced TNF-α mRNA in rat microglial cells [64] and in human peripheral blood mononuclear cells [65]. HU-211, a synthetic cannabinoid, also suppresses TNF-α production in the brain and peripheral blood in rodents with EAE (Fig. **1**) [66].

Nitric oxide (NO) is a short-lived mediator produced by both the isoforms of nitric oxide synthase (NOS), the constitutive (cNOS) and the inducible one (iNOS) found primarily in neurons [67] and astroglial cells [68]. NO generated by iNOS in nanomolar amounts under pathological conditions is a cytotoxic mediator involved in several CNS disorders, including inflammatory, infectious, traumatic and degenerative disease [69, 70]. Excessive release of NO *via* induction of iNOS has been postulated to elicit immune-mediated neurodegenerative inflammatory processes and to cause brain injury in the pathogenesis of MS (Fig. **1**) [71, 72]. It is reported that anandamide inhibits nitric oxide (NO) and TNF-α production by astrocytes stimulated with lipopolysaccharide (LPS) or infected with TMEV [73]. Cannabinoids inhibit NO production by rat microglial cells [74] and LPS-activated mouse macrophages (Fig. **1**) [75, 76]. Inhibiting iNOS expression and NO overproduction in glial cells might be helpful in NO-mediated inflammation leading to neurodegeneration.

HORMONES, NEUROTROPHINS AND CANNABINOIDS

Prolactin (PRL) is a mammotropic neuropeptide produced by pituitary and extra-pituitary cells. The secretion of pituitary PRL is under hypothalamic control. The cytokines IL-1, IL-2, and IL-6 stimulate the production of PRL, while IFN-γ and endothelin-3 are inhibitory. PRL exerts its effects through the PRL receptors (PRLr), which exist as three isoforms. PRL has potent immunomodulatory effects and is structurally related to members of the cytokine/haematopoietic family, while the PRLr is a member of the cytokine/haematopoietic receptor family. Perturbations of PRL physiology have significant immunological effects. Hyper-prolactinemia enhances susceptibility to MS [77]. Before the onset and during EAE, PRL levels were elevated compared with the controls. Accordingly, patients with MS have significantly higher PRL levels than healthy volunteers (Fig. **1**) [78]. Recent data support the concept that the immune system is exposed to a broad framework of regulation, including neuroendocrine control. Interfering with secretion of PRL and glucocorticoids has consistently resulted in a reduction of clinical and pathological manifestations of MS [79]. Previously, it was shown that THC administered into the third cerebral ventricle of male rats lowered plasma PRL concentrations (Fig. **1**) [80]. This effect was exerted within the CNS, as THC did not alter the basal and thyroid-stimulating hormone (TSH)-releasing hormone-induced release of PRL from cultured anterior pituitary (AP) cells [80]. Anandamide and cannabinoids inhibit PRL secretion from the pituitary (Fig. **1**) [80-82]. The study of the relationship among the PRL/PRLr complex, cannabinoids, and the immune system may provide further insights into the potential therapeutic utility of this complex in the treatment of MS.

Leptin is classically considered a hormone through which the hypothalamus senses nutritional state and regulates the balance between food intake and energy expenditure, signalling to the brain the changes in stored energy. Leptin reduces food intake by upregulating appetite-reducing neuropeptides [83, 84] and down-regulating appetite-stimulating factor [85]. Recent evidence has suggested that leptin is involved in autoimmune disease susceptibility. It is known that myelin-reactive Th1 CD4+ cells induce and /or transfer disease and that Th1 cytokines are present in inflammatory EAE lesions in the central nervous system. In contrast, Th2 cytokines are absent and their increase is associated with recovery from EAE or protection from the disease [86]. Leptin is involved in both the induction and progression of EAE in mice [87]. Analysis of the disease susceptibility in naturally leptin-deficient (*ob/ob*) mice before leptin replacement revealed resistance to both active and adoptive EAE that was reversed by leptin administration [87]. Leptin replacement converted Th2- to Th1-type response and shifted IgG antibodies from IgG1 to IgG2a. In addition, leptin administration to susceptible wild-type C57BL/6 J mice worsened the disease by increasing proinflammatory cytokine release and Ig2a production. In addition, it has also been recently observed that a serum leptin surge precedes the onset of EAE in susceptible strain of mice. This peak in serum leptin is correlated with inflammatory anorexia, weight loss, and the development of a pathogenic T cell response against myelin [88]. In animals with EAE, inflammatory brain infiltrates have also been shown to be a source of leptin, attesting to an *in situ* leptin production in active lesions. Systemic and / or *in situ* leptin secretion was not observed in EAE-resistant mice. Taken together, these data show an involvement of leptin in the pathogenesis of central nervous system autoimmunity (Fig. **1**). CB1 receptors and the endocannabinoids anandamide and 2-AG are present in the hypothalamus [89], and anandamide [90, 91] stimulates food intake, that is reduced by SR141716A in wild-type mice but not knockout mice [92]. Defective leptin signalling is associated with elevated hypothalamus, but not cerebellar levels of endocannabinoids in *obese (db/db*) and *ob/ob* mice. Acute leptin treatment of *ob/ob* mice reduces anandamide and 2-AG in the hypothalamus [92]. These findings indicate that endocannabinoids in the hypothalamus may tonically activate CB1 receptors to maintain food intake. Furthermore, physiological concentrations of leptin stimulate the activity of the endocannabinoid-degrading enzyme anandamide hydrolase (fatty acid amide hydrolase, FAAH) in human T lymphocytes. Peripheral lymphocytes of leptin knock-out (*ob/ob*) mice showed decreased FAAH activity and expression, which were reversed to control levels by exogenous leptin [93]. These results suggest that leptin enhances FAAH expression, thus tuning the

immunomodulatory effects of anandamide. The study of the correlation between anandamide and leptin through modulation of circulating leptin levels might be a possible strategy to prevent and eventually treat EAE or other autoimmune diseases.

The neurotrophin nerve growth factor (NGF) is a pivotal molecule in processes that result in an inflammatory hyperalgesia [94, 95]. NGF can also activate neuroimmune systems recognised as cardinal to the inflammatory process [94]. Anandamide and palmitoylethanolamide (PEA) attenuate an NGF-induced hyperalgesia [96]. Such activity is clinically relevant, because currently available nonsteroidal antiinflammatory drugs are ineffective in this model of inflammatory pain [97]. Anandamide is likely to act on neurons expressing CB_1 receptors. Although the effect of PEA is sensitive to the CB_2 receptor antagonist SR144528, the lack of affinity of PEA for the CB_2 receptor obscures the site of action. Furthermore, the efficacy of PEA in the reduction of hyperalgesia offers neuroimmune modulation as a potential site for the development of other novel therapeutic agents devoid of central nervous system-mediated side effects. One of the early symptoms of demyelinating diseases is the altered level of nerve growth factor (NGF). Increase of NGF was found in the optic nerves [98], and in CSF of patients with MS. In rats affected by EAE, the brain tissues contain elevated levels of NGF [99]; furthermore, astrocytes and oligodendrocytes localised in the white matter overexpress NGF mRNA and produce NGF [100]. NGF can act on immune cells through its binding to low affinity NGF receptor and may play an important role in leukocyte-endothelial cell interactions [101]. However, the pathological role of NGF during demyelinating disorders remains to be defined. The study of the relationships among NGF receptors and cannabinoids may provide further insights into the potential therapeutic utility of these substances in the treatment of MS.

THERAPY OF EAE AND CANNABINOIDS

Baker and co-workers using mice with chronic relapsing experimental allergic encephalomyelitis (CREAE), an animal model of MS, have recently presented evidence that both exogenous and endogenous cannabinoids, *via* cannabinoid receptors, alleviate spasticity and tremors [19]. Intravenous administration of THC and also R (+)-WIN552122, a potent synthetic agonist of CB1 and CB2, rapidly decreased both the frequency and amplitude of tremors in limbs and hindlimb spasticity of mice with this disease. Two lines of evidence suggest that these two beneficial effects are mediated by cannabinoid receptors. Firstly, the *S* (-)- enantiomer of WIN552122, and cannabidiol, which are both very weak agonists of CB1 and CB2 receptors, did not reduce spasticity. Secondly, SR141716A, which is a selective CB1 receptor antagonist, and SR144528, which is a selective CB2 receptor antagonist, prevented *R* (1)-WIN552122 from inhibiting tremor. These findings are very important because they may lead to novel strategies for the treatment of MS-induced tremor and spasticity, for which no efficacious remedy has yet been developed. A crucial point that deserves further investigation, at least if therapeutic applications are to be developed, is the full assessment of the possible psychotropic side effects of intravenous administration of cannabinoids. Methanandamide, a CB1-receptor-selective and metabolically stable analogue of the endocannabinoid anandamide [102], was almost as potent as *R* (1)-WIN552122 against hindlimb spasticity in mice with CREAE. This finding implies that drugs based on endocannabinoids, which have been reported to have very low potential for physical dependence [103], could also be used in the treatment of MS-induced spasticity. Another non-psychoactive endogenous compound, the anti-inflammatory mediator PEA [104], also induced a significant, albeit transient inhibition of spasticity [19], however, the mechanism of action of this compound, which does not bind appreciably to CB1 or CB2 receptors, is still a matter of speculation [104]. SR141716A and with less potency SR144528, produced a significant worsening of both tremors and spasticity of hind limbs and tail of CREAE mice [19]. This finding led the authors to raise the possibility that endocannabinoids such as anandamide and 2-AG [102], might be produced during CREAE in an attempt to compensate for the spastic defect. It was shown [20] that in normal ABH mice, whole brains and spinal cords contained similar levels of AEA, 2-AG and PEA. There was a modest increase of AEA in spastic brains compared with levels in normal brains. However, there was a marked increase of AEA, 2-AG and PEA within the spinal cord of spastic mice in comparison with normal animals. On the basis of these findings, it was suggested that augmenting the levels of endogenous AEA might have a therapeutic effect, as exogenously applied and naturally occurring cannabimimetic metabolites, in particular AEA, can limit spasticity. Furthermore, the blockade of degradation with specific inhibitors may represent an alternative to increase the bio-availability of the endocannabinoids. Spasticity could be ameliorated by injection (10 mg/kg i.v.) of either the competitive re-uptake inhibitor AM404 [105] or the selective FAAH inhibitor, AM374 [106], both of which have been shown to enhance AEA neuromodulatory actions [105]. These compounds have very low affinity for cannabinoid receptors [105, 106]. The anti-spastic effect of AM374 (1 mg/kg i.v.) was blocked by cannabinoid receptor antagonists (SR141716A and SR144465, both 5 mg/kg i.v.) administered 20 min prior to AM374. These findings suggest that the inhibitory effect on spasticity by AM374, which does not directly activate CB receptors [106], is due to enhancement of endocannabinoid levels and subsequent stimulation of CB receptors. Both AEA and AM404 may also behave as vanilloid receptor (TRPV1) agonists [107-109], but the role of TRPV1, if any, in control of spasticity is yet to be demonstrated. The extremely selective anandamide transporter inhibitor VDM11 (10 mg/kg i.v.), which has essentially no CB or TRPV1 agonist activity [110], exerts a similar inhibition of spasticity. This finding furthermore supports the hypothesis that endocannabinoids mediate control of spasticity *via* CB receptors. It was observed that agonists of TRPV1 reduce bladder hyper-reactivity in MS [111] and have a modest anti-spastic effect in EAE mice, while a substantial effect was obtained with arvanil, a synthetic compound that can activate both CB1 and TRPV1 receptors [112]. This effect persists using antagonists of CB1 and TRPV1 and in CB1 knockout EAE mice. According to

Table 1. Effect of Cannabinoid Compounds on Immune Response

	SPASTICITY	IMMUNITY
Cannabinoids		
THC	↓ [19]	Suppress T cell proliferation, IFN-γ production, shift the balance of Th1 / Th2 cytokines [55, 61]
CANNABIDIOL	No effect [19]	Increase IL-12 and decrease IL-10 production of murine macrophages [115]
Synthetic cannabinoids		
R(+)-WIN552122	↓ [19]	
S(-)-WIN552122	No effect [19]	
ARVANIL	↓ [120]	Induces apoptosis in Jurkat cells [116]
Endogenous cannabinoids		
ANADAMIDE	↓ [20]	Immuno-regulatory effect on mitogen-induced T and B human lymphocyte proliferation [117]
2-AG	↓ [20]	Dose-related inhibition of T and B cells proliferation [118]
PEA	↓ [20]	Increase AEA-induced microglial cell migration, without affecting other steps of microglial activation, such as proliferation, particle engulfment, and nitric oxide production [119]
Antagonists		
SR141716A	↑ [19]	
SR144528	↑ [19]	
Inhibitors of anandamide inactivation		
AM404	↓ [20]	
AM347	↓ [20]	
Inhibitors of anandamide transporter		
VDM11	↓ [20]	

↓: reduction of spasticity, ↑: increase of spasticity, 1: transient inhibition of spasticity.

Table 2. Receptor Activity of Cannabinoid Compounds

	CB1 receptor activity	CB2 receptor activity	VR1 receptor activity
Cannabinoids			
THC	Partial agonis [121]	Partial agonist [122]	
CANNABIDIOL	No activity [123]	No activity [123]	
Synthetic cannabinoids			
R(+)-WIN552122	Agonist [124]	Agonist [124]	
METHANANDAMIDE[a]	Agonist [125]	Partial agonist [126]	Partial agonist[a] [127]
ARVANIL[b]	Agonist [112]		Agonist [112]
Endogenous cannabinoids			
ANANDAMIDE	Partial agonist [128]	Partial agonist [129]	Partial agonist[a] [127]
2-AG	Agonist [130]	Agonist [131]	
Antagonists			
SR141716A	Antagonist [132]	No activity [132]	
SR144528	No activity [133]	Antagonist [133]	
Inhibitors of anandamide inactivation			
AM404	No activity [105]	No activity [105]	Partial agonist[a] [127]
AM347	No activity [106]	No activity [106]	
Inhibitors of anandamide transporter			
VDM11	No activity [110]	No activity [110]	No activity [110]

a: Methanandamide and AM404 were less potent than AEA at activating VR1, but they are partial agonists when compared with capsaicin.

b: endocannabinoid and vanilloid hybrid.

these experimental findings, it can be suggested that the anti-spastic effect of arvanil can be independent from CB1, CB2 or TRPV1 receptors and mediated by a different site of action. In addition, recent results from our group (see footnote) show that arvanil inhibits cell proliferation and does not induce apoptosis, an effect that is accompanied by decreased levels of IFN-γ. Furthermore, *in vivo* arvanil ameliorates the clinical course of the disease in EAE mice. In Table **1** are summarised the effects of cannabinoid compounds on spasticity and immunity. In Table **2**, we reported the cannabinoid and vanilloid receptor activity of cannabinoid compounds. Further studies can be useful to address if synthetic molecules designed on EC, like arvanil, can improve the disease course in the same animal model. The manipulation of ECs may minimise some of the undesired psychoactive effects associated with CB1 agonism and may have implications for symptom control in MS, opening wider horizons for therapeutic intervention against MS [113, 114].

CONCLUDING REMARKS

There is increasing effort to define the mechanism of the brain-immune interactions. The "immunocannabinoid" system of receptors and endogenous ligands appears to be involved in the modulation of immune and brain system, thus orchestrating the regulation of immunity and chronic inflammatory diseases of the brain. CB1 receptor is expressed in CNS, while CB2 receptor is particularly abundant in immune tissues. Human blood cells express mainly CB2 receptors and most probably CB1 receptors, but considerable work is needed to clarify the distributions of these receptors among the various immune subpopulations. The alteration of various factors can exert a pathogenic role in demyelination in MS. Cytokines, inflammatory mediators, hormones, and neurotrophins are involved in MS susceptibility. Th1 cytokines, and excessive levels of NO, PRL, leptin, and NGF are associated with multiple sclerosis progression.

The image of endocannabinoid system summarised in this review is that of a promising model for the therapy of MS. Cannabinoids and endocannabinoids have been reported to decrease Th1 immunity and inhibit NO, hormones and NGF. *In vivo*, cannabinoids reduce spasticity and pain in EAE models, and increased levels of endocannabinoids are observed during the disease. Exogenously administrated endocannabinoids can limit spasticity, thus suggesting that increasing endocannabinoid levels might represent a therapeutic intervention in MS. The limits to the use of cannabimimetic drugs in MS therapy are associated with their psychoactive effects. The development of new synthetic endocannabinoids could represent an alternative strategy that minimising psychotropic sideeffects could lead to new therapies for the treatment of MS.

FOOTNOTE

Unpublished data submitted for publication:

Arvanil inhibits T lymphocyte activation and ameliorates autoimmune encephalomyelitis.

Malfitano, A.M.; Matarese, G.; Pisanti, S.; Grimaldi, C.; Laezza, C.; Bisogno, T.; Di Marzo, V.; Lechler, R.I.; Bifulco' M.

ABBREVIATIONS

MS = Multiple sclerosis

RR-MS = Relapsing-remitting multiple sclerosis

PP-MS = Primary progressive multiple sclerosis

THC = Δ^9tetrahydrocannabinol

CB = Cannabinoid receptor

2-AG = 2-Arachidonoyl-glycerol

EAE = Experimental autoimmune encephalomyelitis

EC = Endocannabinoids

Th = T helper

TMEV = Theiler's murine encephalomyelitis virus

REFERENCES

[1] Kremenchutzky, M.; Cottrell, D.; Rice, G.; Hader, W.; Baskerville, J.; Koopman, W.; Ebers, G.C. *Brain,* **1999**, *122*, 1941–1950.

[2] Miller, D.H.; Grossman, R.I.; Reingold, S.C.; McFarland, H.F. *Brain,* **1998**, *121*, 3–24.

[3] Losseff, N.A.; Wang, L.; Lai, H.M.; Yoo, D.S.; Gawne-Cain, M.L.; McDonald, W.I.; Miller, D.H.; Thompson, A.J. *Brain,* **1996**, *119*, 2009–2019.

[4] Fox, N.C.; Jenkins, R.; Leary, S.M.; Stevenson, V.L.; Losseff, N.A.; Crum, W.R.; Harvey, R.J.; Rossor, M.N.; Miller, D.H.; Thompson, A.J. *Neurology,* **2000**, *54*, 807–812.

[5] Lassmann, H.; Bru¨ck, W.; Lucchinetti, C. *Trends Mol. Med.,* **2001**, *7*, 115–121.

[6] Compston, A.; Coles A. *Lancet,* **2002**, *359*, 1221-31.

[7] Mechoulam, R.; Gaoni, Y. *Tetrahedron Lett.,* **1967**, *12*, 1109-1111.

[8] Matsuda, L.A.; Lolait, S.J.; Brownstein, M.J.; Young, A.C.; Bonner, T.I. *Nature,* **1990**, *346*, 561-564.

[9] Munro, S.; Thomas, K.L.; Abu-Shaar, M. *Nature,* **1993**, *365*(6441), 61-5.

[10] Matsuda, L.A.; Lolait, S.J.; Brownstein, M.J.; Young, A.C.; Bonner, T.I. *Nature,* **1990**, *346*, 561-564.

[11] Howlett, A.C.; Barth, F.; Bonner, T.I.; Cabral, G.; Casellas, P.; Devane, W.A.; Felder, C.C.; Herkenham, M.; Mackie, K.; Martin, B.R.; Mechoulam, R.; Pertwee, R.G. *Pharmacol. Rev.,* **2002**, *54*, 161-202.

[12] Dewane, W.A.; Hanus, L.; Breuer, A.; Pertwee, R.G.; Stevenson, L.A.; Griffin, G.; Gibson, D.; Mandelbaum, A.; Etinger, A.; Mechoulam, R. *Science,* **1992**, *258*, 1946-49.

[13] Mechoulam, R.; Ben-Shabat, S.; Hanus, L.; Ligumsky, M.; Kaminski, N.E.; Schatz, A.R.; Gopher, A.; Almog, S.; Martin, B.R.; Compton, D.R.; Pertwee, R.G.; Griffin, G.; Bayewitch, M.; Barg, J.; Vogel, Z. *Biochem. Pharmacol.,* **1995**, *50*, 83-90.

[14] Sugiura, T.; Kondo, S.; Sukagawa A.; Nakane, S.; Shinoda, A.; Itoh, K.; Yamashita, A.; Waku, K. *Biochem. Biophys. Res. Commun.,* **1995**, *215*, 89-97.

[15] Deutsch, D.G.; Chin, S.A. *Biochem. Pharmacol.,* **1993**, *46,* 791-796.

[16] Dinh, T.P.; Carpenter, D.; Leslie, F.M.; Freund, T.F.; Katona, I.; Sensi, S.L.; Kathuria, S.; Pomelli, D. *Proc. Natl. Acad. Sci.,* **2002**, *99,* 10819-24.

[17] Kreitzer, A.C.; Regehr, W.G. *Neuron,* **2001**, *29*, 717-27.

[18] Wilson, R.I.; Nicoll, R.A. *Nature,* **2001**, *410*, 588-92.

[19] Baker, D.; Pryce, G.; Croxford, J.L.; Brown, P.; Pertwee, R.G.; Huffman, J.W.; Layward, L. *Nature,* **2000,** *404*, 84-87.

[20] Baker, D.; Pryce, G.; Croxford, J.L.; Brown, P.; Pertwee, R.G.; Makriyannis, A.; Khanolkar, A.; Layward, L.; Fezza, F.; Bisogno, T.; Di Marzo V. *FASEB J.,* **2001**, *15*(2), 300-302.

[21] Consroe, P.; Musty, R.; Rein, J.; Tillery, W.; Pertwee, R. *Eur. Neurol.,* **1997**, *38*, 44-8.

[22] Killestein, J.; Hoogervorst, E.L.; Reif, M.; Kalkers, N.F.; Van Loenen, A.C.; Staats, P.G.; Gorter, R.W.; Uitdehaag, B.M.; Polman, C.H. *Neurology,* **2002**, *58*, 1404-7.

[23] Robson, P.J.; Wade, D.T.; Makela, P.M; House, H. In: *12th Annual Symposium on the Cannabinoids. Burlington (VT): International Cannabinoid Research Society;* **2002**. *pp. 56*. Available from:

www.cannabinoidsociety.org/progab2.pdf. Results of recent phase III trials available from: www.gwpharm.com/news_pres_05_nov_02.html

[24] Vaney, C.; Jobin, P.; Tscopp, F.; Heinzel, M.; Schnelle, M. In: *12th Annual Symposium on the Cannabinoids. Burlington (VT): International Cannabinoid Research Society;* **2002**. *pp. 57*. Available from: www.cannabinoidsociety.org/progab2.pdf

[25] Twitchell, W.; Brown, S.; Mackie, K. *J. Neurophysiol.,* **1997***, 78*, 43-50.

[26] Hampson, A.J.; Grimaldi, M.; Axelrod, J.; Wink, D. *Proc. Natl. Acad. Sci. USA,* **1998***, 95,* 8268-73.

[27] Pitt, D.; Werner, P.; Raine, C.S. *Nat. Med.,* **2000***, 6*, 67-70.

[28] Smith, T.; Groom, A.; Zhu, B.; Turski, L., *Nat. Med.,* **2000***, 6*, 62-6.

[29] Kapoor, R.; Davies, M.; Blaker, P.A.; Hall, S.M., Smith, K.J. *Ann. Neurol.,* **2003***, 53*, 174-80.

[30] Di Marzo, V.; Breivogel, C.S.; Tao, Q.; Bridgen, D.T.; Razdan, R.K.; Zimmer, A.M.; Zimmer, A.; Martin, B.R. *J. Neurochem.,* **2000***, 75*, 2434-44.

[31] Breivogel, C.S.; Griffin, G.; Di Marzo, V.; Martin, B.R. *Mol. Pharmacol.,* **2001***, 60*, 155-63.

[32] Monory, K. ; Tzavara, E.T. ; Lexime, J. ; Ledent, C.; Parmentier, M.; Borsodi, A.; Hanoune, J. *Biochem. Biophys. Res. Commun.,* **2002***, 292*, 231-5.

[33] Ledent, C.; Valverde, O.; Cossu, G.; Petitet, F.; Aubert, J.F. Beslot, F.; Bohme, G.A.; Imperato, A.; Pedrazzini, T.; Roques, B.P.; Vassart, G.; Fratta, W.; Parmentier, M. *Science,* **1999***, 283*, 401-4.

[34] Zimmer, A.; Zimmer, A.M.; Hohmann, A.G.; Herkenham, M.; Bonner, T.I. *Proc. Natl. Acad. Sci.USA,* **1999***, 96*, 5780-85.

[35] Hillard, C.J.; Campbell, W.B. *J. Lipid Res.,* **1997***, 38*, 2383-98.

[36] Di Marzo, V. *Biochem. Biophys. Acta,* **1998***, 1392*, 153-75.

[37] Di Marzo, V. *Biochem. Biophys. Acta,* **1998***, 1392*, 153-75.

[38] Di Marzo, V.; Melck, D.; Bisogno, T.; De Petrocellis, L. *Trends Neurosci.,* **1998***, 21*, 521-28.

[39] Dijkstra, C.D.; van der Voort, E.R.; De Groot, C.J.; Huitinga, I.; Uitdehaag, B.M.; Polman, C.H.; Berkenbosch, F. *Psychoneuroendocrinology,* **1994***, 19*, 135-42.

[40] Gade-Andavolu, R.; MacMurray, J.P.; Blake, H.; Muhleman, D.; Tourtellotte, W.; Comings, D.E. *Arch. Neurol.,* **1998***, 55*, 513-16.

[41] Wekerle, H.; Kojima K.; lannes-Vieira J. Lassmann H., Linington C. *Ann. Neurol.,* **1994***, 36*, S47-S53.

[42] Bourrie, B.; Bribes, E.; Esclangon, M.; Garcia, L.; Marchland J.; Thomas, C.; Maffrand, J.P.; Casellas, P. *Proc. Natl. Acad. Sci. USA,* **1999***, 96*, 12855-12859.

[43] Zamvil, S.S.; Steinman, L. *Ann. Rev. Immunol.,* **1990***, 8*, 579-621.

[44] Rochen, M.; Racke, M.; Shevach, E.M. *Immunol. Today,* **1996***, 17*, 225-231.

[45] Panitch, H.S.; Hirsch, R.L.; Schindler J.; Johnson K.P. *Neurology,* **1987***, 37,* 1097-1103.

[46] Bouaboula, M.; Rinaldi, M.; Carayon, P.; Carillon, C.; Delpech, B.; Shire, D.; Le Fur, G.; Casellas, P. *Eur. J. Biochem.,* **1993***, 214*(1), 173-80.

[47] Daaka, Y.; Klein, T.W.; Friedman, H. *Adv. Exp. Med. Biol.,* **1995***, 373*, 91-6.

[48] Schatz, A.R.; Lee, M.; Condie, R.B.; Pulaski, J.T.; Kaminski, N.E. *Toxicol. Appl. Pharmacol.,* **1997***, 142*(2), 278-87.

[49] Noe, S.N.; Newton, C.; Widen, R.; Friedman, H.; Klein, T.W. *J Neuroimmunol.,* **2000***, 110*(1-2), 161-7.

[50] Brown, S.M.; Wager-Miller, J.; Macie, K. *Biochim Biophys Acta,* **2002***, 1576*(3), 255-64.

[51] Galiegue, S.; Mary, S.; Marchand, J.; Dussossoy, D.; Carriere, D.; Carayon, P.; Bouaboula, M.; Shire, D.; Le Fur, G.; Casellas, P. *Eur. J. Biochem.,* **1995***, 232*(1), 54-61.

[52] Lee, S.F.; Newton, C.; Widen, R.; Friedman, H.; Klein, TW. *Eur. J. Pharmacol.,* **2001***, 423*(2-3), 235-41.

[53] Klein, T.W.; Newton, C.; Friedman, H. *Immunol. Today,* **1998***, 19*(8), 373-81.

[54] Newton, C.A.; Klein, T.W.; Friedman, H. *Infect. Immun.,* **1994***, 62,* 4015-20.

[55] Klein, T.W.; Newton, C.A.; Nakachi, N.; Friedman, H. *J. Immunol.,* **2000***, 164,* 6461-66.

[56] Zhu, L.X.; Sharma, S.; Stolina, M.; Gardner, B.; Roth, M.D.; Tashkin, D.P.; Dubinett, S.M. *J. Immunol.,* **2000***, 165,* 373-80.

[57] Smith, S.R.; Terminelli, C.; Denhardt, G. *J. Pharmacol. Exp. Ther.,* **2000***, 293, 136-50.*

[58] Ouyang, Y; Hwang, S.G.; Han, S.H.; Kaminski, N.E. *Mol. Pharmacol.,* **1998**, *53,* 676-83.

[59] Hemmer, B.; Cepok, S., Nessler, S.; Sommer, N. *Curr. Opin. Neurol.,* **2002***, 1, 227–231.*

[60] Yuan, M.; Kiertscher, S.M.; Cheng, Q.; Zoumalan, R.; Tashkin, D.P.; Roth, M.D. *J. Neuroimmunol.,* **2002***, 133*(1-2), 124-31.

[61] Navikas, V.; Link, H. *J. Neurosci. Res.,* **1996***, 45*, 322-333.

[62] Molina-Holgado, F.; Molina-Holgado, E.; Guaza, C. *FEBS Lett.,* **1998***, 433*, 139-142.

[63] Bitsch, A.; Kuhlmann, T.; Da Costa, C.; Bunkowski, S.; Polak, T.; Bruck, W. *Glia,* **2000***, 29*, 366-375.

[64] Puffenbarger, R.A.; Boothe, A.C.; Cabral, G.A. *Glia,* **2000***, 29*, 58-69.

[65] Berdyshev, E.; Boichot, E.; Germain, N.; Allain, N.; Anger, J.P.; Lagente, V. *Eur. J. Pharmacol.,* **1997***, 330*, 231-240.

[66] Achiron, A.; Miron, S.; Lavie, V.; Margalit, R.; Biegon, A. *J. Neuroimmunol.,* **2000***, 102*, 26-31.

[67] Knowles, R.G.; Palacios, M., Palmer, R.M.J.; Moncada, S. *Proc. Natl Acad. Sci. USA,* **1989***, 86*, 5159-5162.

[68] Salter, M.; Knowles, R.M.; Moncada, S. *FEBS Lett.,* **1991***, 291*, 145-149.

[69] Dawson, V.L.; Dawson, T.M. *Proc. Soc. Exp. Biol. Med.,***1996***, 211*, 33-40.

[70] Bolanos, J.P.; Almeida, A.; Stewart, V.; Peuchen, S.; Land, J.M.; Clark, J.B.; Heales, S.J.R. *J. Neurochem.,* **1997***, 68*, 2227-2240.

[71] Santiago, E.; Perez-Mediavilla, L.A.; Lopez-Moratalla, N. *J. Physiol. Biochem.,* **1998***, 54*, 229-237.

[72] Pozza, M.; Bettelli, C.; Aloe, L.; Giardino, L.; Calza, L. *Brain Res.,* **2000***, 855*, 39-46.

[73] Molina-Holgado, F.; Lledo, A.; Guaza, C. *Neuroreport,* **1997***, 8*, 1929-1233.

[74] Waksman, Y.; Olson, J.M.; Carlisle, S.J.; Cabral, G.A. *Pharmacol. Exp. Ther.,* **1999***, 288*, 1357-1366.

[75] Coffey, R.G.; Yamamoto, Y.; Snella, E.; Pross, S. *Biochem. Pharmacol.,* **1996,** *52*, 743-751.

[76] Jeon, Y.J.; Yang, K.H.; Pulaski, J.T.; Kaminski, N.E. *Mol. Pharmacol.,* **1996***, 50*, 334-341.

[77] Chikanza, I.C. *Ann. N.Y. Acad. Sci.,* **1999,** *876*, 119-130.

[78] Azar, S.T.; Yamout, B. *Endocr. Res.,* **1999,** *25*, 207-214.

[79] Dijkstra, C.D.; Polman, C.H.; Berkenbosch, F. *Trends Pharmacol. Sci.,* **1993,** *14*, 124-129.

[80] Rettori, V.; Wenger, T.; Snyder, G.; Salterio, S.; McCann, S.M. *Neuroendocrinology,* **1988,** *47*(6), 498-503.

[81] Wenger, T.; Moldrich, G. *Prostaglandins Leukot. Essent. Fatty Acids,* **2002***, 66*(2-3) 301-7.

[82] Fernandez-Ruiz, J.J.; Munoz, R.M.; Romero, J.; Villanua, M.A.; Makriyannis, A.; Ramos, J.A. *Biochem. Pharmacol.,* **1997***, 53*(12), 1919-27.

[83] Schwartz, M.W.; Seeley, R.J.; Woods, S.C.; Weigle, D.S.; Campfield, L.A.; Burn, P.; Baskin, D.G. *Diabetes,* **1997***, 46*(12), 2119-23.

[84] Seeley, R.J.; Yagaloff, K.A.; Fisher, S.L.; Burn, P.; Thiele, T.E.; van Dijk, G.; Baskin, D.G.; Schwartz, M.W. *Nature,* **1997***, 390*(6658), 349-51.

[85] Stephens, T,W.; Basinski, M.; Bristol, P.K.; Bue-Valleskey, J.M.; Burgett, S.G.; Craft, L.; Hale, J.; Hoffmann, J.; Hsiung, H.M.; Kriauciunas, A.; Mackellar, W.; Rosteck Jr. R.P; Schoner, B.; Smith, D.; Tinsley, C.F.; Zhang, X.-Y.; Heiman, M. *Nature,* **1995***, 377*(6549),530-2.

[86] Steinman, L. *Neuron,* **1999***, 24*(3), 511-4.

[87] Matarese, G.; Di Giacomo, A.; Sanna, V.; Lord, G.M.; Howard, J.K.; Di Tuoro, A.; Bloom, S.R.; Lechler, R.I.; Zappacosta, S.; Fontana, S. *J. Immunol.,* **2001***, 166*(10), 5909-16.

[88] Sanna, V.; Di Giacomo, A.; La Cava, A.; Lechler, R.I.; Fontana, S.; Zappacosta, S.; Matarese, G. *J. Clin. Invest.,* **2003**, *111*(2):241-50.

[89] Gonzalez, S.; Manzanares, J.; Berrendero, F.; Wenger, T.; Corchero, J.; Bisogno, T.; Romero, J.; Fuentes, J.A.; Di Marzo, V.; Ramos, J.A.; Fernandez-Ruiz, J. *Neuroendocrinology,* **1999***, 70*(2), 137-45.

[90] Williams, C.M.; Kirkham, T.C. *Psychopharmacology,* **1999***, 143*(3), 315-7.

[91] Hao, S.; Avraham, Y.; Mechoulam, R.; Berry, E.M. *Eur. J. Pharmacol.,* **2000***, 392*(3), 147-56.

[92] Di Marzo, V.; Goparaju, S.K.; Wang, L.; Liu, J.; Batkai, S.; Jarai, Z.; Fezza, F.; Miura, G.I.; Palmiter, R.D.; Sugiura, T.; Kunos, G. *Nature,* **2001***, 410*(6830), 822-5.

[93] Maccarrone, M.; Di Rienzo, M.; Finazzi-Agro, A.; Rossi, A. *J. Biol. Chem.,* **2003***, 278*(15), 13318-24.
[94] McMahon, S.B.; Bennett, D.L.H. Growth factors and pain, The Pharmcology of Pain. Ed.; Dickenson, A.H.; Besson, J.M.: Berlin, Springer, **1997**, pp. 135-57.
[95] Shu, X-Q.; Mendell, L.M. *Proc. Natl. Acad. Sci. USA,* **1999***, 96*, 7693-6.
[96] Farquhar-Smith, W.P.; Rice, A.S. *Anesthesiology,* **2003***, 99*(6), 1391-401.
[97] Amann, R.; Schuligoi, R.; Herzeg, G.; Donnerer, J. *Pain,* **1995***, 64*, 323-9.
[98] Micera, A.; Lambiase, A.; Rama, P.; Aloe, L. *Mult. Scler.,* **1999,** *5*, 389-394.
[99] Micera, A.; De Simone, R.; Aloe, L. *Arch. Ital. Biol.,* **1995,** *133*, 131-142.
[100] Micera, A.; Vigneti,E.; Aloe, L. *Exp. Neurol.,* **1998***, 154*, 41-46.
[101] Nataf, S.; Naveilhan, P.; Sindji, L.; Darcy, F.; Brachet, P.; Montero-Menei, C.N. *J. Neurosci. Res.,* **1998,** *52*, 83-92.
[102] Mechoulam, R.; Fride, E.; Di Marzo, V. *Eur. J. Pharmacol.* **1998***, 359*, 1-18.
[103] Aceto, M.D.; Scates, S.M.; Razdan, R.K.; Martin, B.R. *J. Pharmacol. Exp. Ther.,* **1998***, 287*(2), 598-605.
[104] Lambert, D.M.; Di Marzo, V. *Curr. Med. Chem.,* **1999***, 6*(8), 757-773.
[105] Beltramo, M.; Stella, N.; Calignano, A.; Lin, S.Y.; Makriyannis, A.; Piomelli, D. *Science,* **1997***, 277*, 1094-1097.
[106] Deutsch, D.G.; Lin, S.; Hill, W.A.; Morse, K.L.; Salehani, D.; Arreaza, G.; Omeir, R.L.; Makriyannis, A. *Biochem. Biophys. Res. Commun*., **1997***, 231***,** 217–221.
[107] Zygmunt, P.M.; Petersson, J.; Andersson, D.A.; Chuang, H.; Sorgard, M.; Di Marzo, V.; Julius, D.; Hogestatt, E.D. *Nature,* **1999***, 400,* 452–447.
[108] Smart, D.; Jerman, J.C. *Trends Pharmacol. Sci.,* **2000***, 21*, 134.
[109] Smart, D.; Gunthorpe, M,J.; Jerman, J.C.; Nasir, S.; Gray, G.; Muir, A.I.; Chambers, J.K.; Randell, A.D.; Davis, J.B. *Br. J. Pharmacol.,* **2000,** *129*, 227-230.
[110] De Petrocellis, L.; Bisogno, T.; Davis, J.B.; Pertwee, R.G.; Di Marzo V. *FEBS Lett*., **2000,** *483,* 52–56.
[111] Fowler, C.J.; Jewkes, D.; McDonald, W.I.; Lynn, B.; de Groat, W.C. *Lancet***, 1992***, 339* (8803), 1239.
[112] Melck, D.; Bisogno, T.; De Petrocellis, L.; Chuang, H.; Julius, D.; Bifulco, M.; Di Marzo V. *Biochem. Biophys. Res. Commun.,* **1999***, 262*, 275-284.
[113] De Petrocellis, L.; Melck, D.; Bisogno, T.; Di Marzo, V. *Chem. Phys. Lipids,* **2000***, 108*, 191-209.
[114] Di Marzo, V.; Bisogno, T.; De Petrocellis, L. *Curr. Pharm. Des.,* **2000***, 6*, 1361-1380.
[115] Sacerdote, P.; Martucci, C.; Vaccani, A.; Bariselli, F.; Panerai, A.E.; Colombo, A.; Parolaro, D.; Massi, P. *J. Neuroimmunol.,* **2005***, 159*(1-2), 97-105.
[116] Rancho, R.; de la Vega, L.; Appendino, G.; Di Marzo, V.; Macho, A.; Munoz, E. *Br. J. Pharmacol.,* **2003***, 140*(6),1035-44.
[117] Schwarz, H.; Blanco, F.J.; Lotz, M. *J. Neuroimmunol.,* **1994***, 55*(1), 107-15.
[118] Lee, M.; Yang, K.H.; Kaminski, N.E. *J. Pharmacol. Exp. Ther.,* **1995***, 275*(2), 529-36.
[119] Franklin, A.; Parmentier-Batteur, S.; Walter, L.; Greenberg, D.A., Stella, N. *J. Neurosci.,* **2003***, 23*(21), 7767-75.
[120] Brooks, J.W.; Pryce, G.; Bisogno, T.; Jaggar, S.I.; Hankey, D.J.; Brown, P.; Bridges, D.; Ledent, C.; Bifulco, M.; Rice, A.S.; Di Marzo, V.; Baker, D. *Eur. J. Pharmacol.,* **2002***, 439*(1-3), 83-92.
[121] Gerard, C.M.; Mollereau, C.; Vassart, G.; Parmentier, M. *Biochem. J.,* **1991***, 279*, 129–134.
[122] Bayewitch, M.; Rhee, M.H.; Avidor- Reiss, T.; Breuer, A.; Mechoulam, R.; Vogel, Z. *J. Biol. Chem.,* **1996***, 271*, 9902–9905.
[123] Showalter, V.M.; Compton, D.R.; Martin, B.R.; Abood, M.E. *J. Pharmacol. Exp. Ther.,* **1996**, 278, 989–999.
[124] Tao, Q.; Abood, M.E. *J. Pharmacol. Exp. Ther.,* **1998***, 285*, 651–658.
[125] Lin, S.; Khanolkar, A.D.; Fan, P.; Goutopoulos, A.; Qin, C.; Papahadjis, D.; Makriyannis, A. *J. Med. Chem.,* **1998***, 41*, 5353–5361.
[126] Berglund, B.A.; Boring, D.L.; Wilken, G.H.; Makriyannis, A.; Howlett, A.C.; Lin, S. *Prostaglandins Leukot. Essent. Fatty Acids,* **1998***, 59*, 111–118.
[127] Roberts, L.A.; Christie, M.J.; Connor, M. *Br. J. Pharmacol.,* **2002***, 137*(4), 421-8.
[128] Bayewitch, M.,; Avidor-Riess, T.; Levy, R.; Barg, J.; Mechoulam, R.; Vogel, Z. *FEBS Lett.,* **1995***, 375*, 143–147.
[129] Rinaldi-Carmona, M.; Pialot, F.; Congy, C.; Redon, E.; Barth, F.; Bachy, A.; Breliere, J.C.; Soubrie, P.; Le Fur, G. *Life Sci.,* **1996***, 58*, 1239–1247.
[130] Stella, N.; Schweitzer, P.; Piomelli, D. *Nature,* **1997***, 388*, 773–778.
[131] Sugiura, T., Kondo, S., Kishimoto, S.; Miyashita, I.; Nakane, S.; Kodata, T.; Suhera, T.; Takayama, H.; Waku, K. *J. Biol. Chem.,* **2000***, 275*, 605–612.
[132] Rinaldi-Carmona, M.; Barth, F.; Heaulme, M.; Shire, D.; Calandra, B.; Congy, C.; Martinez, S.; Maruani, J.; Neliat, G.; Caput, D.; Ferrara, P.; Soubrie, P.; Breliere, J.C.; Lefur, G. *FEBS Lett.,* **1994***, 350*, 240–244.
[133] Rinaldi-Carmona, M.; Barth, F.; Millan, J.; Derocq, J.M.; Casellas, P.; Congy, C.; Oustric, D.; Sarran, M.; Bouaboula, M.; Calandra, B.; Portier, M.; Shire, D.; Breliere, J.C.; Le Fur, G.L. *J. Pharmacol. Exp. Ther.,* **1998***, 284*(2), 644-50.