Molecular Neurobiology

ISSN0893-7648/98/24(1–3): 29–52/$15.25

Cannabinoids and Neuroprotection

Robert I. Grundy, Monica Rabuffetti, and Massimiliano Beltramo*

Schering Plough Research Institute, San Raffaele Science Park, Via Olgettina 58, Milan, Italy

Abstract

Cannabinoid compounds are endowed with pharmacological properties that make them interesting candidates for therapeutic development. These properties have been known since antiquity. However, in the last decade extremely important advances in the understanding of the physiology, pharmacology, and molecular biology of the cannabinoid system have given this field of research fresh impetus and have renewed the interest in the possible clinical exploitation of these compounds. In the present review we summarize the effects elicited, at the cellular level, by cannabinoids acting through receptor-dependent and receptor-independent mechanisms. These data suggest different ways by which cannabinoids may act as neuroprotective agents (prevention of excitotoxicity by inhibition of glutamate release, antioxidant effects, anti-inflammatory actions, etc.). The experimental evidence supporting these hypotheses are presented and discussed with regard to both preclinical and clinical studies in disease states such as cerebral ischemia, brain trauma, and Multiple Sclerosis.

Index Entries: Cerebral ischemia; brain trauma; multiple sclerosis; cannabinoid receptor; signaling pathways; neuroprotection.

Introduction

The term cannabinoids (CB) refers to a heterogenous group of natural, synthetic, and endogenous compounds. The first naturally occuring cannabinoid, cannabidiol, was isolated in 1942 *(1)* from hashish, a preparation obtained from the resin produced by the female flowers of the plant *Cannabis sativa.* Hydrogenated derivatives of cannabidiol, tetrahydrocannabinols, were synthetized and shown to posses psychoactive properties *(1),* but only two decades later delta-9-tetrahydrocannabinol (THC) was identified as the main psychoactive component of *Cannabis sativa* preparations *(2).* Cannabinoids modulate a series of important physiological functions ranging from locomotor activity to memory, from pain perception to food intake, from inflammatory reaction to cancer development. In the 1980s, their clinical potential enticed several groups to work on the synthesis of potent agonist compounds

* Author to whom all correspondence and reprint requests should be addressed. E-mail: massimiliano.beltramo@spcorp.com or robert.grundy@spcorp.com

(WIN55212, CP55940, HU-210) with the aim of testing their therapeutic usefulness. Despite the fact that the search for effective medicines was not wholly successful, thanks to these compounds it was possible to make substantial progress in understanding the pharmacological actions of cannabinoids. The first direct evidence for the existence of a cannabinoid receptor came from binding studies using the synthetic cannabinoid agonist [^{3}H]CP55,940 *(3)*. A few years later, the cloning of the rat CB1 receptor *(4)* confirmed the presence of a diffusely distributed cannabinoid system in the central nervous system (CNS). This discovery, together with that of the first endogenous ligand for the CB1 receptor *(5)*, a lipid molecule christened anandamide (arachidonylethanolamide), gave new impetus to research in the cannabinoid field. This renewed effort led, in the following years, to a series of relevant findings: the identification of a second cannabinoid receptor, called CB2, mostly localized in immune system cells *(6)*, and the synthesis of specific CB1 (SR141716A *[7]*) and CB2 (SR144528 *[8]*) antagonists. A second endogenous cannabinoid, 2-arachidonoyl glycerol, was also discovered in both the gut *(9)* and the CNS *(10,11)*. Investigation into the formation and inactivation pathways of endogenous ligands, focusing mainly on anandamide, unveiled the biosynthetic route involved in anandamide production and some of the mechanisms involved in its regulation *(12,13)*. The inactivation pathways of anandamide were also intensively studied and shown to be similar to that of classical neurotransmitters, i.e., mediated by a membrane transporter *(14–16)* followed by the action of a degrading enzyme, known either as anandamide amido hydrolase or fatty acid amide hydrolase *(17–19)*. Experiments involving the blockade of the inactivation pathway resulted in an augmentation of the effect elicited by anandamide highlighting its importance in modulating anandamide signaling *(15,20)*. Selective agonists for both CB1 (ACEA *[21]*) and CB2 (L-759656 *[22,23]*; HU-308 *[24]*) receptor have been synthetized endowing pharmacologists with new tools to explore cannabinoid function. The burgeoning research in the field has not only brought a better understanding of the mechanisms elicited by cannabinoids, but has also refreshed the interest in the possible therapeutic application of cannabinoids. Several recent reviews have been dedicated to various aspects of the potential therapeutic application of cannabinoids *(25–29)*. The present article reviews the evidence supporting a neuroprotective effect of cannabinoids in neurodegeneration, an emerging area of potential clinical interest that has so far received little attention.

Neuroprotective Mechanisms

Neurodegeneration is a complex phenomenon involving several different mechanisms that have been only partially revealed. In the case of progressive neurodegenerative pathologies such as Parkinson's disease (PD), Progressive Supranuclear Palsy, Alzheimer's disease (AD), Hungtington's disease (HD), Amiotrophic Lateral Sclerosis (ALS), Multiple Sclerosis (MS), and so on, though several hypotheses have been proposed to explain the causes responsible for neurodegeneration, none has obtained a general consensus. Conversely, for acute neurodegenerative pathologies, such as ischemic damage, the general framework of events responsible in neurodegeneration has been more clearly established. For example the principal mechanism involved in ischemia is energy failure, which leads to production of reactive oxygen radicals, depolarization of cells followed by massive release of excitatory amino acids, elevated calcium influx in the postsynaptic cells and ensuing disregulation of cell homeostasis.

Another mechanism potentially linked to various chronic and acute neurodegenerative pathologies is the activation of inflammatory processes. Inflammation could induce neurodegeneration either through the release of toxic mediators or through the activation of an autoimmune response against brain antigens, as in the case of MS.

The neuroprotective effects ascribed to cannabinoid action are quite varied. The first

part of the review illustrates the effects elicited by cannabinoids at the cellular level; the second part examines the experimental and clinical evidence in favor of a neuroprotective effect of cannabinoids in specific neuropathologies and relates these results to the possible cellular mechanisms involved.

Receptor-Mediated Mechanisms

To date, two cannabinoid receptors have been characterized: 1) the cannabinoid receptor 1 (CB1) primarily expressed in tissues of the CNS *(4,30,31)* and 2) the cannabinoid receptor 2 (CB2) mainly expressed in the immune system *(6,32).* The CB1 is a prime target for the psychoactive effect of cannabinoids, whereas cannabinoid-induced immunomodulation is predominantly CB2-mediated. There exists evidence, however, to suggest that cannabinoids may act at sites other than the two established receptors *(33,34),* leading to speculation of the existence of further cannabinoid receptors and to the identification of nonreceptor-mediated effects.

Action Related to CB1 Activation

The structure and functional expression of the CB1 receptor was first described by Matsuda et al. *(4).* Since the discovery of the CB1 receptor, its localization in the CNS has been described (using radioligand autoradiography, *in situ* hybridization, and immunocytochemistry), in the cortex, striatum, hippocampus, amygdala, hypothalamus, cerebellum, brain stem, and spinal cord of both rodents and human *(30,35–43).* Peripheral expression has been documented in nerve terminals *(44,45),* and in non-neural tissues, including the testis *(46),* endothelial cells *(47),* smooth-muscle cells *(48),* and, albeit at a much lower level then the CB2 receptor, in immune system cells *(32).* Activation of CB1 receptors represents the predominant signaling mechanism for endogenous and exogenous cannabinoids in the brain and spinal cord.

Over the past 10 years, an enormous amount of work has been carried out in order to elucidate the mechanism by which the CB1 receptor transduces its signals (Fig. 1). Prior to the characterization of specific cannabinoid receptors, Howlett et al. reported that the cannabimimetic inhibition of adenylate cyclase could be blocked by pertussis toxin *(49),* therefore implicating G_i proteins in the mechanism of cannabinoid signaling. Much work has since been published confirming the role of G_i protein activation in CB1 signaling *(50–52),* although a number of reports also suggest that stimulation of the CB1 receptor could lead to activation of G_s protein under particular conditions *(53–56).*

As a consequence of the activation of G_i protein, CB1 receptor stimulation reduces adenylate cyclase activity and therefore decreases cAMP levels *(49,57).* The modulation of cAMP is considered the major signaling pathway of the CB1 receptor.

In addition to the adenylate cyclase-mediated activity elicited by CB1, several other pathways have been proposed as possible mediators of cannabinoid signaling. The activity of mitogen-activated protein (MAP) kinases p38 and p44/42 has been shown to be increased after stimulation of CB1 in Chinese hamster ovary (CHO) cells transfected with CB1 *(58,59),* and in human vascular endothelial cells *(60).* This CB1 mediated activation of MAP kinases has been proposed to be via two possible mechanisms. Sanchez and co-workers proposed that MAP kinase activation is caused either by a $G_i\beta\gamma$ subunit activation of the small G protein Raf-1, which in turn activates MAP kinase or via a non-G_i mediated activation involving the hydrolysis of sphyngomyelin *(61).* In addition, other stress-activated protein kinases have been implicated in CB1 receptor signaling, these include extracellular-related kinase and c-Jun N-terminal kinase *(60).* Protein kinase B/Akt has also been implicated in CB1 signaling via activation of phosphoinositide 3′-kinase in CB1-transfected CHO cells *(62)* as has the zinc finger-related gene *krox-24* in the same cell line *(63).* Anandamide has also been demonstrated to trigger phosphorylation of focal adhesion kinase *(64),* which may lead to neurotrophic effects. Furthermore, in astro-

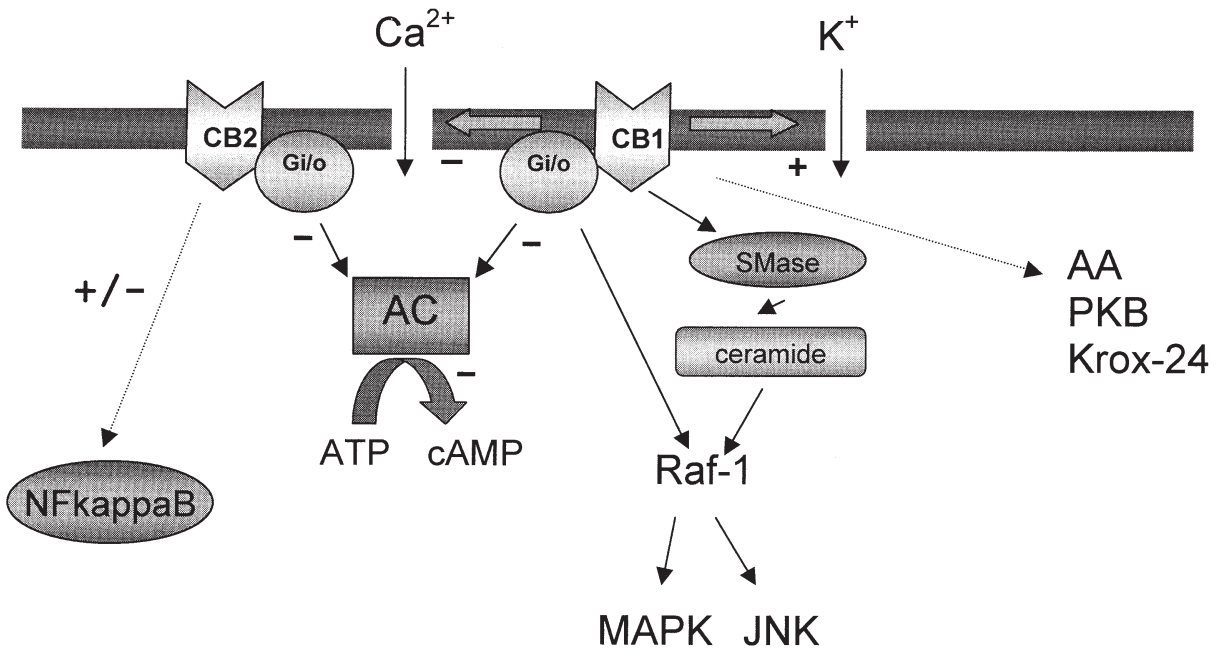

Fig. 1. Mechanisms of cannabinoid receptor signaling. Endogenous and exogenous cannabinoids activate cannabinoid receptors located at the plasma membrane. CB1 signaling occurs predominantly via activation of $G_{i/o}$ protein, which leads to an inhibition of adenylate cyclase (AC), leading subsequently to an inhibition of the conversion of adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP). Activation of the CB1 receptor also leads to an inhibition of Ca^{2+} channels and an activation of K^+ channels, as well as an activation of RAF-1 via either the β subunit of the $G_{i/o}$ protein or through a sphingomyelinase (SMase)/ceramide pathway, leading to activation of mitogen-activated protein kinase (MAPK) and c-Jun N terminal kinase (JNK). CB1 activation has also been reported to lead to the production of arachidonic acid (AA), activation of protein kinase B (PKB), and expression of the immediate early gene *Krox-24.* Activation of the CB2 receptor leads to activation of $G_{i/o}$ protein as in the case of CB1 activation, and consequent inhibition of AC and reduction of cAMP levels. It is also thought to lead to the inhibition of nuclear factor-κB (NFκB), although an increase or a decrease in its activity has been described depending on the experimental conditions and timing.

cytes activation of CB1 has been shown to increase the release of arachidonic acid *(65).*

The link between G proteins and the CB1 receptor takes an intriguing turn in light of work revealing that CB1 can sequester G-proteins from a common pool, therefore making them unavailable to couple to other receptors *(66).* An interesting perspective on CB1 signaling is also revealed by studies indicating an effect of the G protein subunit composition on the efficacy and potency of CB1 receptor activation *(67),* suggesting an increasing complexity in the central cannabinoid receptor-signaling mechanism.

The modulation of ion-channel currents is a second major consequence of CB1 receptor activation. Activation of the CB1 receptor results in inhibition of N type calcium currents in neuroblastoma-glioma cells *(68)* and rat superior cervical ganglion neurons *(69),* Q-type calcium currents in murine tumor line AtT20 cells *(51),* and L-type calcium currents in arterial smooth-muscle cells *(70).* Moreover, activation of CB1 has also been shown to activate inwardly rectifying potassium channels in murine tumor line AtT20 cells *(51)* and in Xenopus oocyte *(71).* In addition, the ability of the CB1 receptor to modulate I_A potassium currents has also been

demonstrated in hippocampal culture *(72–74),* and has been shown to decrease potassium M- and K-currents via CB1 receptors in hippocampal neurons *(55,75).*

CB1 activation influences not only the intracellular homeostasis, but also the intercellular comunication, and the modulation of neurotransmitter release is an important consequence. Several studies indicate that CB1 activation decreases the release of glutamate, a prominent neurotransmitter in neurodegeneration (*see* section on cerebral ischemia, in vitro studies). Cannabinoids have also been shown to inhibit GABAergic transmission in several brain areas *(76–81).* The release of nitric oxide (NO), which plays a key role in neurodegenerative processes, is again affected by the cannabinoid system, with cannabinoids inhibiting the production of NO in microglia *(82),* astrocytes *(83),* neurones *(84),* and macrophages *(85).*

In addition, activation of CB1 influences dopaminergic transmission in striatal slices *(86)* and in vivo *(20,87),* inhibits noradrenaline release in isolated rat atria and vasa deferentia *(44),* inhibits acetylcholine release in vivo *(88–90)* and in a synaptosome preparation *(91),* and inhibits serotonin release in mouse brain cortical slices *(92).*

Action Related to CB2 Activation

Considering that inflammation plays an important role in the pathology of most neurodegenerative diseases, the ability to control the degree of inflammatory reaction would be advantageous. The potential participation of the CB2 receptor in this process may represent a mechanism by which therapeutic intervention could be achieved.

Compared to CB1 receptor signaling pathways those of the CB2 receptor have been investigated less thoroughly, (Fig. 1) whereas much attention has been paid to its control of immune-cell interaction and cytokine production. Responses of the CB2 receptor also seems to depend on the cannabinoid agonist used, with different cannabinoid agonists sometime eliciting opposite effects (*see* Table 1).

There is little evidence of CB2 receptor presence in the CNS. Skaper and colleagues reported that the CB2 receptor is expressed on granule cells and Purkinje cells of the cerebellum *(93).* More recently using the fluorescence-activated cell sorting (FACS) technique, CB2 immunoreactivity has been observed in isolated dorsal-root ganglia neurons and F-11 cell line, a dorsal-root ganglion x neuroblastoma hybridoma *(94).* The CB2 receptor mRNA has also been detected in cultured rat microglial cells, where it has been shown to be negatively coupled to adenylyl cyclase activity *(95).* This result is intriguing since it suggests the possibility that cannabinoids may affect microglial function in the CNS.

Conversely, the CB2 receptor is abundant in immune tissues, in particular in spleen macrophage and in tonsils, where the mRNA content is equivalent to that of CB1 in the CNS. Among the main human blood cell subpopulations, CB2 mRNA is expressed at high levels in B cells and natural killer (NK) cells, but is also present in monocytes, neutrophils, and finally T cells *(32).* CB2 receptor, like the CB1 receptor, signals via $G_{i/o}$ proteins, inhibiting adenylate cyclase activity *(96–99)* and increasing MAP kinase activity *(100).* In spleenocytes, CB2 receptor stimulation by cannabinol reduces cAMP levels, which correlates with a decline in PKA activity that in turn would be responsible for a decrease in the activation of the CREB/ATF family of transcription factors *(101).*

Using Affymetrix DNA chips and Western-blot analysis to study the gene- and protein-expression profile of HL-60 cells transfected with CB2 and stimulated by CP55940, it was possible to show a biphasic regulation of IκB-α expression by cannabinoid receptor *(102).* An early decrease of IκB-α protein, due to degradation, was followed by an increase of IκB-α gene expression and protein levels. This observation suggests an involvement of the CB2 receptor in the regulation of the NF-κB transcription factor machinery *(101–103).* Conversely, in spleenocytes and thymocytes, cannabidiol was reported to inhibit the NFκB/c-Rel pathway *(101)* at early time post-adminis-

Table 1
Cannabinoid Effects on Cytokine

Experimental model	Challenge	Treatment	Cytokine	Effect	Antagonism	References
In vitro (cell type)						
Macrophage cell line RAW364.7	None	Δ^9-THC (10^{-7}–$10^{-5}M$)	TNF-α (active form)	↓	N.T.	*(110)*
Murine peritoneal macrophages	None	Δ^9-THC	IL-1; TNF-α	↑	N.T.	*(115)*
Rat cortical microglial cells	LPS (1—1000 ng/mL)	Δ^9-THC (0.1–10 μM)	IL-1α; IL-1β; TNF-α; IL-6 (mRNA)	↓	None	*(112)*
HL-60	None	CP55940 (10 nM)	IL-8; MCP-1 (mRNA, protein)	↑	SR144528 (200 nM)	*(116)*
HL-60	None	CP55940 (10 nM)	IL-8; MCP-1; MIP-1β; TNF-α	↑	SR144528 (200 nM)	*(102)*
Murine peritoneal macrophages	LPS (10 μg/mL)	Δ^9-THC (3–10 μg/mL)	IL-1α; IL-1β	↑	N.T.	*(113)*
Murine astrocytes	TMEV (10^5 PFU/well)	Anandamide (1–25 μ*M*)	IL-6	↑	SR141716A (1 μ*M*)	*(175)*
In vivo (species)						
Mouse	*Legionella pneumophila*	Δ^9-THC (8 mg/kg twice)	TNF-α; IL-6 (blood levels)	↑	N.T.	*(114)*
Mouse	LPS	WIN55212-2 (3.1–50 mg/kg)	TNF-α; IL-10 (serum levels)	↓	SR141716A (25 and 100 mg/kg)	*(111)*

N.T., not tested; LPS, lipopolysaccharide; TMEV, Theiler's murine encephalomyelitis virus.

tration and similar results were obtained with THC in the macrophage cell line RAW264.7 *(98).* The endogenous cannabinoid 2-AG was reported to have a modest inhibitory effect on NFκB/Rel on splenocytes, whereas it exerted a stronger suppression of NF-AT *(103).* Thus, further studies will be necessary to clarify the relevance of the difference obtained using different agonists and different cell lines.

Interacting with most cells of the immune system, natural and synthetic cannabinoids are able to modulate multiple immune responses, both in humans and rodents *(104).* Cannabinoids can inhibit T-lymphocyte functions such as proliferation and cytotoxicity *(105–107),* decrease antibody formation by B cells *(104),* and affect production of several cytokines, mostly decreasing interleukin-2 (IL-2), interferon-γ (IFN-γ), and IL-12 and increasing the levels of IL-4 and IL-10 *(104,107).* Macrophage functions are also affected; several studies on rat and mouse peritoneal macrophages and cultured cell lines showed that various CB receptor ligands suppress important functions such as phagocytosis, cytolysis, antigen presentation, and protein expression *(104,106,108).* Evidence for the involvement of CB2 receptor in the regulation of immune functions also comes from CB2 knockout mice. Using a T-cell co-stimulation assay, Buckley and colleagues observed that THC inhibits helper T-cell activation through macrophages derived from wild-type, but not from knockout mice. Central effects of cannabinoids were not altered in these mice, thus indicating that this effect is mediated by the CB2 receptor *(109).*

Cannabinoids could exert an effect on macrophage/microglial cells by modulating acute-phase cytokine release, mainly IL-1, tumor necrosis factor-α (TNF-α), and IL-6, which play a major role in the development of damage in neurodegenerative/neuroinflammatory conditions, such as cerebral ischemia. There are many reports regarding the effects of both natural and synthetic cannabinoids on these cytokines, but, depending on the model system, these results are often conflicting, and the involvement of cannabinoid receptors is unclear (*see* Table 2). Δ^9-THC has been demonstrated to suppress tumoricidal activity of RAW264.7, a macrophage cell line, at least in part by decreasing the intracellular conversion to the active form of the cytotoxic cytokine TNF-α *(110).* More recently, Smith and colleagues observed that in mice challenged with lipopolysaccharide (LPS), two cannabinoid agonists, WIN55212-2 and HU-210, decreased serum TNF-α. This effect were antagonized by SR141716A, but not by SR144528, thus indicating a role for the CB1 receptor subtype in cytokine modulation *(111).* Δ^9-THC treatment appears to be able to reduce mRNAs for IL-1α, IL-1β, TNF-α, and IL-6, in LPS-treated rat microglial cells, but, this effect is apparently not mediated through either CB1 or CB2 cannabinoid receptors *(112).* Conversely, other reports suggest that acute-phase cytokines are induced by cannabinoid treatment. In 1994, Zhu and colleagues demonstrated that THC facilitates IL-1α and -β release using macrophage culture stimulated with the microbial inflammatory substance LPS *(113).* In addition, Klein and colleagues showed that, in mice infected with *Legionella pneumophila,* blood levels of TNF-α and IL-6 were significantly elevated by THC treatment *(114).* The same researchers also found that in cultured peritoneal macrophages THC treatment increased IL-1 and TNF-α production *(115).* The most obvious explanations for the opposite responses observed are: the nature of the challenge used (inflammatory such as the LPS or viral such as the Theiler's murine encephalomyelitis virus [TMEV]), the concentration of the cannabinoid and finally, the system used, which can vary considerably from one paper to another. The combination of these factors provide a possible explanation for the observed variability in response to cannabinoid treatment. Taken together, these results, although contradictory, suggest that the cannabinoid system influences the functioning of the cytokine network, but a thorough understanding of these complex mechanisms requires further investigation.

CB2 receptor may also be involved in leukocyte recruitment to the site of inflammation

Table 2
Cannabinoids and Experimental Models of Neurodegeneration

Experimental conditions	Protective cannabinoid	Mechanism	References
In vitro			
Glutamate excitotoxicity	HU-211 (5—10 μ*M*)	NMDA antagonism	*(125,159)*
	Palmitoylethanolamide (100 μ*M*)	Unknown	*(93)*
	WIN 55212-2 (100 nM)	CB1 agonism	*(160)*
	Cannabidiol (10 μ*M*), THC (10 μ*M*)	Anti-oxidation	*(146)*
Hypoxia	WIN 55212-2 (3—100 nM)	CB1 agonism	*(161)*
	Anandamide (30—300 nM)	Unknown	*(162)*
	2-arachidonylglycerol (300—3000 nM)	Unknown	*(162)*
In vivo			
Experimental allergic encephalomyelitis	Δ^9-THC (5—25 mg/kg/d)	N.I.	*(177)*
	Δ^8-THC (40 mg/kg/d)	N.I.	*(178)*
	HU-211 (5 mg/kg)	NMDA antagonism	*(179)*
	WIN 55212-2, (5 mg/kg)	CB1 and CB2 agonism	*(180)*
	Methanandamide (0.05 mg/kg)	N.I.	*(180)*
	JWH-133 (1.5 mg/kg)	N.I.	*(180)*
	Anandamide (10 mg/kg)	N.I.	*(181)*
	2-arachidonylglycerol (10 mg/kg)	N.I.	*(181)*
	Palmitoylethanolamide (10 mg/kg)	N.I.	*(181)*
Brain trauma	HU-211 (4.4—25 mg/kg)	NMDA antagonism	*(124,172,173)*
Cerebral ischemia	HU-211 (4—8 mg/kg)	NMDA antagonism	*(163—168)*
	WIN 55212-2 (1 mg/kg)	CB1 agonism	*(161)*
	Δ^9-THC (0.1—10 mg/kg)	N.I.	*(169)*

N.I., not identified.

and injury. In 1999, Jbilo et al., using nucleic acid microarray assays, showed for the first time that very low concentrations of CP55940 upregulate the expression of two different chemokines, IL-8 and MCP-1, in the promyelocytic cell line HL60 transfected with the CB2 receptor *(116).* Activation of the gene transcription for these two chemokines is followed by enhanced expression and secretion of the two proteins and these effects are abolished by the CB2 antagonist SR144528. Indeed, IL-8 is an important chemotactic factor for neutrophils *(117),* whereas MCP-1 is mainly involved in the attraction of monocytes *(118).* On the other hand, it has been shown that CP55940 is able to induce a dose-dependent inhibition of both spontaneous migration and formyl-metionyl-leucine-phenylalanine (FMLP)-induced chemotaxis in macrophages *(119).* Both CB1 and the CB2 antagonists were able to reverse CP55940 effects on spontaneous migration, although the CB2 antagonist was more potent. Conversely, only the CB2 antagonist was able to reverse both in vitro and in vivo FMLP-induced chemotaxis. These data indicate that the effects of cannabinoids on macrophage locomotion and chemotaxis are predominantly CB2-mediated.

The variety of cellular types utilized (macrophage, macrophage cell line, astrocyte, microglia, HE-60, etc.), the use of different types of stimuli to induce particular cellular state (LPS and TMEV), and not least the range of cannabinoid concentrations (from nM to μM) used to perform the experiments may contribute to the different and sometime

contradictory results obtained. Further studies, using more standardized conditions, would be necessary to help clarify this complex issue.

Thus, the effect of cannabinoids on the immune system are of potential interest regarding the induction of neuroprotection through reduction of inflammation. CB2 receptor-mediated neuroprotection would offer the considerable advantage of avoiding the psycotropic side effects elicited by CB1 receptor activation. However, it is currently unclear weather agonists or antagonists would be better suited for this purpose.

NMDA-Mediated Neuroprotection

Dexanabinol (HU-211), [(+)-(3S,4S)-7-hydroxy-Δ^6-tetrahydrocannabinol-1, 1-dimethylheptyl] is a synthetic compound structurally related to cannabinoids. It does not possess a cannabinoid pharmacological profile, displays a very low affinity to the cannabinoid receptors and is inactive as a cannabimimetic in vivo *(120,121).* However, pharmacological studies carried out in vivo and in vitro indicate that HU-211 antagonizes glutamatergic neurotransmission in the brain and describes HU-211 as an NMDA non-competitive antagonist *(122).* This cannabinoid stereospecifically blocks the NMDA receptor by interacting with a site close to, but distinct from, that of non-competitive antagonists, such as MK-801 and phencyclidine, and from the recognition sites of glutamate, glycine, and polyamines *(123).* Recent investigations suggest that HU-211 acts as a neuroprotective agent in several models of acute neurodegeneration (*see* section on experimental and clinical evidence of neuroprotection) via blockade of NMDA-mediated Ca^{2+} influx into neurons *(124)* and through antioxidative mechanisms *(125).*

It has been reported that anandamide also acts on NMDA receptor by modulating its activity in a manner that is not mimicked by THC and is unaffected by cannabinoid receptor antagonists *(126).*

Vanilloid Receptor

Increasing experimental evidence supports the notion that the endocannabinoid anandamide, and some of its analogs (methanandamide, palmitoylethanolamide, oleoylethanolamide, dihomo-γ-linolenylethanolamide and docosatetraenylethanolamide), interact with the vanilloid receptor VR1. The first evidence concerning this possible interaction came from the observation of striking similarities amongst anandamide transporter inhibitors, such as AM404, and vanilloid receptor ligands, such as olvanil *(127,128).* Indeed the vanilloid receptor agonist olvanil is able to inhibit anandamide transport in the CCF-STTG-1 human astrocytoma cell line *(128).* It was then suggested that anandamide could induce vasodilation by directly activating the vanilloid receptor *(129)* and it has been subsequently demonstrated that anandamide acts as a full agonist of the human vanilloid receptor *(130).* The debate about the possibility of anandamide acting as an endogenous ligand for the VR1 receptor has been particularly animated *(131–134).* It must also be borne in mind that cannabinoids and vanilloids often exert opposing pharmacological effects (e.g., analgesia vs hyperalgesia). However, the relevance of the vanilloid system in modulating the putative neuroprotective effects of endocannabinoids remain to be explored. Nevertheless, a recent finding suggests a dual effect of anandamide on the survival of a neural cell line, it being pro-apoptotic through activation of VR1 receptor and anti-apoptotic via stimulation of the CB1 receptor *(135).*

Other Receptors

Some studies have also reported the interaction of anandamide with both muscarinic *(136)* and serotoninergic receptors *(137).*

Another effect of anandamide that deserves attention and is not mediated by known receptors is the inhibition of the function of gap junctions. Anandamide, but not WIN-55212-2 and CP55940, has been reported to inhibit gap-junction communication in astrocyte culture

originating from the striatum (only a marginal effect was observed in cultures obtained from other brain regions) *(138).* The effect of anandamide was not reversed by the selective CB1 antagonist SR141716A, however, it was sensitive to pertussis toxin, suggesting a receptor-mediated mechanism *(138).* The absence of CB1 immunoreactivity in astrocytes *(41,42,139)* and lack of detectable binding for [^{3}H]SR141716A in astrocyte cultures *(139),* further supports the hypothesis that gap-junction inhibition by anandamide is not mediated by cannabinoid CB1 receptors. However, recently observation at the ultrasctructural level using an anti-CB1 antibody showed the presence of CB1-like immunoreactivity in striatal astrocytes *(140).* In light of these new results, further studies will be necessary to clarify if CB1 is present in astrocytes or if the antibody cross-reacts with a CB1-like receptor. Considering that the regulation of gap-junction function has been proposed to have an important role in ischemic and hypoxic cell injury *(141–143),* the actions of cannabinoids on gap junctions would seem to be worthy of further investigation.

Receptor-Independent Mechanisms

With the identification of selective agonists and antagonists it has been possible to ascribe most of the effects induced by cannabinoids to their actions on CB1, CB2, or other receptors. Nonetheless, there is a body of experimental evidence that cannot be explained by the interaction of cannabinoids with receptors. These data suggest the existence of other mechanisms involved in cannabinoid effects. The two most investigated receptor-independent mechanisms of cannabinoids are their antioxidant properties and their capacity to alter cell-membrane properties.

Antioxidant Properties

The study of oxidative stress as an etiologic agent of neurodegenerative disease is an area attracting growing attention. Oxidative stress has been suggested as a possible common cause for neurodegenerative diseases by several authors (*144* and references therein). In normal conditions, the production of reactive oxygen species (ROS) is a strictly controlled process *(145).* Oxidative stress is observed when the normal balance between oxidative events and antioxidant mechanisms is disrupted. The presence of uncontrolled amounts of ROS leads to the derivatization of lipids, proteins, and nucleic acids. These chemical reactions induce effects ranging from cell-cycle alteration to changes in membrane ion homeostasis and could rapidly lead to cell demise.

In the last decade, the neuroprotective properties of antioxidant molecules have been tested quite extensively, leading to some encouraging results *(145).* However, only recently it has been suggested that cannabinoids may also act through an antioxidant mechanism *(125,146).* The first evidence of the antioxidant properties of cannabinoids came from the study of the neuroprotective effect of HU-211 on neuronal cultures subjected to oxidative stress with sodium nitroprusside *(125).* Cyclic voltametry data confirm that HU-211 has an oxidation potential similar to that of known antioxidants (i.e., butylhydroxytoluene), and other cannabinoids (cannabidiol, THC, cannabinol, nabilone, and levonantradol) also display similar oxidation profiles *(125,146).* It is worth noting that the concentration required for an antioxidant effect are in the micromolar range for all of the tested compounds, which is higher than that required to activate cannabinoid receptors.

The endocannabinoid anandamide has no antioxidant properties *(146).* However, its unsaturated congener N-palmitoylethanolamide has been shown to inhibit in vitro free radical-induced oxidation of lipids *(147).* This compound is produced through the same pathway involved in anandamide biosythesis and is co-released with anandamide in neuronal cell cultures stimulated with ionomycin *(14).* N-palmitoylethanolamide has been suggested to act through the CB2 *(93)* or a CB-like receptor *(148),* however, its antioxidant effects in vivo have not been studied.

Alteration of Membrane Properties

In the 1970s and 1980s, before the discovery of the cannabinoid receptors, there was an ongoing debate about the possibility that cannabinoids could exert their effects through nonreceptor-mediated mechanisms. This hypothesis mainly derived support from the high lipophilicity of cannabinoid compounds that would allow them to easily integrate into the lipid bilayer of the cell membrane. However, studies on the relationship between the lipophilicity and the pharmacological activity of several cannabinoids showed the absence of a positive correlation, suggesting that the lipophilicity is not a primary determinant of the cannabinoids pharmacological activity *(149).*

Experimental and Clinical Evidence of Neuroprotection

In light of the diverse range of actions mediated by cannabinoids on both neural cells and peripheral immune cells, an increasing amount of work is being carried out to investigate the neuroprotective properties of these compounds. What follows is an attempt to summarize the work done in vitro and in vivo on a range of clinically relevant experimental models of neurodegeneration (*see* Table 2).

Cerebral Ischemia

Cerebral ischemia is a neurodegenerative condition that is currently without a satisfactory clinical treatment and is the third leading cause of death in Western countries.

In Vitro Studies

Glutamate Excitotoxicity

Crucial to the ability of cannabinoids to afford neuroprotection is the influence of CB1 receptor activation on neurotransmitter release. Glutamate has been shown to play a critical role in the development of neurotoxicity *(150)* and indeed a clear link can be observed between CB1 receptor activation and a decrease in glutamatergic transmission. This effect has been observed in the rat in cortical neurons *(151),* neurons of the substantia nigra *pars reticulata (152),* periaqueductal gray *(153),* cerebellar Purkinje cells *(154),* hippocampus *(155–157),* and dorsal striatum *(158).*

Glutamate-mediated excitotoxicity has been widely studied as an in vitro model in which to investigate potentially neuroprotective compounds. The nonpsychotropic, NMDA receptor-inhibiting cannabinoid HU-211 was found to protect primary cultured rat forebrain neurons against exposure to glutamate, NMDA, and quisqualate *(123,159).* In a model of glutamate-induced cell death in cerebellar granule cells, the endocannabinoid palmitoylethanolamide and other cannabinoids were also shown to increase neuronal survival *(93).* In addition, in rat hippocampal neurons, WIN55212-2 completely blocked calcium spiking and prevented neuronal death induced by low extracellular magnesium concentration, a model of cell death that results in excessive activation of NMDA receptors. This effect was reversed by the CB1 antagonist SR1417161A *(160).* Conversely, the neuroprotective actions of cannabidiol and THC were not reversed by SR141716A and were attributed to their antioxidant effects following exposure of primary rat cortical cells to glutamate *(146).*

Hypoxia

In an attempt to reflect the oxygen deprived environment experienced by neuronal cells during cerebral ischemia, Greenberg and co-workers used an in vitro model of hypoxia and glucose deprivation in cultured cortical neurons to demonstrate that the synthetic cannabinoid WIN 55252-2 *(161)* and the endocannabinoids anandamide and 2-arachidonylglycerol afford neuroprotection, an effect not reversed by cannabinoid receptor antagonists *(162).*

In Vivo Studies

HU-211 is effective in treating rats and gerbils against global ischemia *(163,164)* and is capable of reducing forebrain ischemia in rats *(165)* when administered up to 60 min post-ischemia. In addition, HU-211 has been shown

to protect against focal ischemia when administered up to 70 min post-occlusion *(166–168).*

Preliminary data also suggest that Δ^9-THC could afford a neuroprotective effect against forebrain cerebral ischemia in rat, when administered daily for 7 d prior to occlusion *(169).*

Recently, a report has been published demonstrating that WIN55212-2, a CB1-CB2 cannabinoid receptor agonist, protects against a four-vessel occlusion in rat models of global ischemia when administered 40 min prior to occlusion *(161).* The same study also reported a time-course of the neuroprotective effects in a model of permanent ischemia induced by middle cerebral artery occlusion, suggesting a time window of efficacy of up to 30 min post-ischemia. The neuroprotective effect was abolished in both instances by the CB1 receptor antagonist SR141716A, suggesting the involvement of a CB1 receptor-mediated mechanism. No major changes in blood pressure or body temperature, which could at least partially account for this effect, were observed. It is important to note that several cannabinoids have been shown to cause hypotension in the rat and hypothermia in rodents via CB1-mediated mechanisms *(170,171)* and it is possible that this CB1-mediated increase in blood flow or decrease in brain temperature could provide a neuroprotective effect after experimental cerebral ischemia.

Clinical Evidence

Prompted by the promising results obtained in preclinical models with HU-211 Pharmos Corporation has completed a Phase I clinical trial in stroke demonstrating the safety of this compound (http://www.pharmoscorp.com/product/dexanabinol.htm).

Brain Trauma

Brain trauma is the major cause of mortality in young people in the Western world and the only accepted treatment is administration of steroidal anti-inflammatory drugs.

In Vitro Studies

Glutamate-mediated excitotoxcity is thought to be a major mediator of cell death resulting from head injury. Therefore, the in vitro excitotoxic studies described in the section regarding cerebral ischemia are also relevant in brain trauma.

In Vivo Studies

HU-211 has proved effective in improving motor *(172)* and memory functions *(173)* after closed head injury in rat. Additional studies have clarified that its neuroprotective effect is probably due to an attenuation of calcium levels *(124)* and to a reduction in levels of mediators of cell damage such as TNF-α *(173),* levels of which are also reduced by HU-211 in brain homogenates of ischemic rats *(168).*

Clinical Evidence

HU-211 has been proved to be well-tolerated *(174)* and a Phase II clinical trial has already been completed. The results of this trial showed that elevation of intracranial pressure was prevented by dexanabinol and that a single intravenous injection of 200 mg of dexanabinol resulted in a better Glasgow Outcome Scale at 1 mo compared to placebo-treated patients. Enrollment of patient for a Phase III trial is planned in seven European countries and in Israel (http://www.pharmoscorp.com/product/dexanabinol.htm).

Multiple Sclerosis

MS is a severely debilitating autoimmune disease that involves the demyelination of nerves throughout the brain and spinal cord.

In Vitro Studies

Due to the complex pathology of MS, there are very few reports of in vitro paradigms involving this disease and the potential therapeutic effects of cannabinoids. However, it has been reported that in a cell-culture preparation of astrocytes from mice infected with observed TMEV, a virus thought to cause effects that

resemble MS, anandamide potentiated the production of IL-6, a potentially anti-inflammatory cytokine *(175)* and suppressed the production of the pro-inflammatory cytokine TNF-α and NO *(83).* Nevertheless, given the difficulty in modeling the complex relationship that exist between diverse cell types in MS, caution must be exercised in interpreting in vitro results.

In Vivo Studies

Experimental autoimmune encephalomyletis (EAE) is a T cell-mediated, autoimmune disorder characterized by CNS inflammation and demyelination, features reminiscent of the human disease MS. As such, EAE has been studied in animals in order to elucidate the mechanisms of, and develop potential treatments for, MS *(176).* Due to the immunomodulatory properties of cannabinoids, research efforts to evaluate their potential have focused on this disease state over the past 10 years. The cannabinoid Δ^9-THC was found to dramatically increase survival after EAE inoculation in rats and guinea pigs *(177).* The less psychotropic analogue of Δ^9-THC, Δ^8-THC was also found to reduce the incidence and severity of neurological deficit in two strains of rat *(178).* Recently the nonpsychotropic cannabinoid HU-211 was discovered to be effective in limiting the effects of EAE in the rat *(179).* These data, coupled with data from elegant studies performed by Baker et al., which demonstrated that several cannabinoids could control spasticity and tremor in a rat EAE model via a cannabinod receptor-mediated mechanism *(180,181),* indicate that the cannabinoid system could be an important target for the development of an effective treatment for MS.

Clinical Evidence

Numerous anecdotal accounts of the therapeutic benefits of illegal self-medication with cannabis with regard to MS exist *(182),* and are reinforced by a handful of clinical accounts of the beneficial effects of specific cannabinoids. Administration of a dose as low as 5 mg of THC have been reported to improve muscle spasticity *(183)* and tremor *(184).* Nabilone, a synthetic THC analogue, at a dose of 1 mg was reported to improve general well-being, reduce muscle spasms, and reduce frequency of nocturia *(185).* A questionnaire sent to MS suffers showed that a large proportion of them reported improvement of several symptoms (such as spasticity, chronic pain, tremor, etc.) after cannabis use *(186).* Despite this encouraging evidence, clinical trails with suitable patient numbers have not yet been performed. In the absence of such a study, no firm clinical conclusions can be drawn on the effect of cannabinoids on MS at present.

Parkinson/Alzheimers Disease

Cannabinoids have been shown to modulate the dopaminergic system *(86),* and numerous reports demonstrate the impairment of memory formation by cannabinoids *(187).* The potential involvement of cannabinoids in PD model has been investigated in preclinical studies leading to the discovery that CB1 receptor mRNA expression is under negative influence of dopamine-mediated events in the caudate putamen *(188),* and increases in 6-hydroxydopamine-lesioned rats both with *(189)* and without L-DOPA treatment *(190).* Although no evidence exists of cannabinoids playing a direct role in the neurodegeneration responsible for PD and AD, it is conceivable that the cannabinoid system may play some role in both pathologies. On the other hand the potential of this system as an adjunct to existing therapies for PD has been suggested *(191),* but a small clinical study carried out to test the effects of marijuana on Parkinsonian tremor led to negative results *(192).*

Conclusions and Perspectives

There is a rapidly growing amount of evidence suggesting that cannabinoids may be neuroprotective in different experimental con-

ditions, and the initiation of clinical trials to prove their therapeutic efficacy in humans illustrates the interest that this aspect of cannabinoid pharmacology has stimulated. Although there is an abundance of data supporting a neuroprotective role for cannabinoids, it must be borne in mind that some studies have demonstrated neurotoxic effects after cannabinoid administration. In cultured primary hippocampal cells, Δ^9-THC was found to be neurotoxic *(193),* as was anandamide, which was found to induce apoptosis in PC-12 cells *(194).* Consequently the potential neurotoxicity of some cannabinoids must be considered when progressing with potential therapeutic strategies.

Interestingly, most of the results on neuroprotection are based on the effect induced by actions on CB1 or NMDA receptors. Other targets such as the CB2 receptor, endogenous cannabinoid inactivation system, and biosynthetic pathways have not attracted the same attention aimed at investigating and evaluating their neuroprotective potential. To exploit these targets, further studies are needed to elucidate some crucial aspects of their biology. The opposing data obtained with the activation of the CB2 receptor on the production of inflammatory agents and activation of leukocytes must be clarified before a valid anti-inflammatory approach to immune-response modulation and neuroprotection can be followed up. On the other hand, the cloning of the enzyme involved in the biosynthesis of the endogenous cannabinoids and of the transporter implicated in their inactivation will represent an important step towards understanding the feasibility of a neuroprotective strategy aimed at interfering with their function. Nevertheless, they remain interesting therapeutic targets and could offer the important advantage of being devoid of the psychotropic effects elicited by direct activation of the CB1 receptor, effects that in the past have rendered the development of cannabinoid medicines difficult. Thus, the study of cannabinoids in neuroprotection has great potential for further development, which, it is hoped, will not be hampered by the political and social concerns about the use of cannabis and its derivatives as medicine.

Acknowledgments

We would like to thank Drs. Julian Romero and Nephi Stella for their helpful comments.

References

1. Adams R. (1942) Marihuana. *Harvey Lectures* **37,** 168–197.
2. Gaoni Y. and Mechoulam R. (1964) Isolation, structure and partial synthesis of an active constituent of hashish. *J. Am. Chem. Soc.* **86,** 1646–1647.
3. Devane W. A., Dysarz F. A., Johnson M. R., Melvin L. S., and Howlett A. C. (1988) Determination and characterization of a cannabinoid receptor in rat brain. *Mol. Pharmacol.* **34,** 605–613.
4. Matsuda L. A., Lolait S. J., Brownstein M. J., Young A. C., and Bonner T. I. (1990) Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature* **346,** 561–564.
5. Devane W. A., Hanus L., Breuer A., Pertwee R. G., Stevenson L. A., Griffin G., et al. (1992) Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* **258,** 1946–1949.
6. Munro S., Thomas K. L., and Abu-Shaar M. (1993) Molecular characterization of a peripheral receptor for cannabinoids. *Nature* **365,** 61–65.
7. Rinaldi-Carmona M., Barth F., Heaulme M., Shire D., Calandra B., Congy C., et al. (1994) SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. *FEBS Lett.* **350,** 240–244.
8. Rinaldi-Carmona M., Barth F., Millan J., Derocq J. M., Casellas P., Congy C., et al. (1998) SR 144528, the first potent and selective antagonist of the CB2 cannabinoid receptor. *J. Pharmacol. Exp. Ther.* **284,** 644–650.
9. Mechoulam R., Ben-Shabat S., Hanus L., Ligumsky M., Kaminski N. E., Schatz A. R., et al. (1995) Identification of an endogenous 2-

monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem. Pharmacol.* **50,** 83–90.
10. Sugiura T., Kondo S., Sukagawa A., Nakane S., Shinoda A., Itoh K., et al. (1995) 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. *Biochem. Biophys. Res. Commun.* **215,** 89–97.
11. Stella N., Schweitzer P., and Piomelli D. (1997) A second endogenous cannabinoid that modulates long-term potentiation. *Nature* **388,** 773–778.
12. Cadas H., Gaillet S., Beltramo M., Venance L., and Piomelli D. (1996) Biosynthesis of an endogenous cannabinoid precursor in neurons and its control by calcium and cAMP. *J. Neurosci.* **16,** 3934–3942.
13. Cadas H., di Tomaso E., and Piomelli D. (1997) Occurrence and biosynthesis of endogenous cannabinoid precursor, N-arachidonoyl phosphatidylethanolamine, in rat brain. *J. Neurosci.* **17,** 1226–1242.
14. Di Marzo, V, Fontana A., Cadas H., Schinelli S., Cimino G., Schwartz J. C., and Piomelli D. (1994) Formation and inactivation of endogenous cannabinoid anandamide in central neurons. *Nature* **372,** 686–691.
15. Beltramo M., Stella N., Calignano A., Lin S. Y., Makriyannis A., and Piomelli D. (1997) Functional role of high-affinity anandamide transport, as revealed by selective inhibition. *Science* **277,** 1094–1097.
16. Hillard C. J., Edgemond W. S., Jarrahian A., and Campbell W. B. (1997) Accumulation of N-arachidonoylethanolamine (anandamide) into cerebellar granule cells occurs via facilitated diffusion. *J. Neurochem.* **69,** 631–638.
17. Deutsch D. G. and Chin S. A. (1993) Enzymatic synthesis and degradation of anandamide, a cannabinoid receptor agonist. *Biochem. Pharmacol.* **46,** 791–796.
18. Desarnaud F., Cadas H., and Piomelli D. (1995) Anandamide amidohydrolase activity in rat brain microsomes. Identification and partial characterization. *J. Biol. Chem.* **270,** 6030–6035.
19. Cravatt B. F., Giang D. K., Mayfield S. P., Boger D. L., Lerner R. A., and Gilula N. B. (1996) Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. *Nature* **384,** 83–87.
20. Beltramo M., de Fonseca F. R., Navarro M., Calignano A., Gorriti M. A., Grammatikopoulos G., et al. (2000) Reversal of dopamine D(2) receptor responses by an anandamide transport inhibitor. *J. Neurosci.* **20,** 3401–3407.
21. Hillard C. J., Manna S., Greenberg M. J., DiCamelli R., Ross R. A., Stevenson L. A., et al. (1999) Synthesis and characterization of potent and selective agonists of the neuronal cannabinoid receptor (CB1). *J. Pharmacol. Exp. Ther.* **289,** 1427–1433.
22. Gareau Y., Dufresne C., Gallant M, Rochette C., Sawyer N., Slipetz D. M., et al. (1996) Structure activity relationship of tetrahydrocannabinol analogues on human cannabinoid receptors. *Bioorg. Med. Chem. Lett.* **6,** 189–194.
23. Ross R. A., Brockie H. C., Stevenson L. A., Murphy V. L., Templeton F., Makriyannis A., and Pertwee R. G. (1999) Agonist-inverse agonist characterization at CB1 and CB2 cannabinoid receptors of L759633, L759656, and AM630. *Br. J. Pharmacol.* **126,** 665–672.
24. Hanus L., Breuer A., Tchilibon S., Shiloah S., Goldenberg D., Horowitz M., et al. (1999) HU-308: a specific agonist for CB(2), a peripheral cannabinoid receptor. *Proc. Natl. Acad. Sci. USA* **96,** 14,228–14,233.
25. Di Marzo, V., Melck D., De Petrocellis L., and Bisogno T. (2000) Cannabimimetic fatty acid derivatives in cancer and inflammation. *Prostaglandins Other Lipid Mediat.* **61,** 43–61.
26. Kunos G., Jarai Z., Varga K., Liu J., Wang L., and Wagner J. A. (2000) Cardiovascular effects of endocannabinoids-the plot thickens. *Prostaglandins Other Lipid Mediat.* **61,** 71–84.
27. Piomelli D., Giuffrida A., Calignano A., and Rodriguez de Fonseca F. (2000) The endocannabinoid system as a target for therapeutic drugs. *Trends Pharmacol. Sci.* **21,** 218–224.
28. Pertwee R. G. (2000) Cannabinoid receptor ligands: clinical and neuropharmacological considerations, relevant to future drug discovery and development. *Expt. Opin Investig. Drugs* **9,** 1553–1571.
29. Pertwee R. G. (2001) Cannabinoid receptors and pain. *Prog. Neurobiol.* **63,** 569–611.
30. Herkenham M., Lynn A. B., Johnson M. R., Melvin L. S., de Costa B. R., and Rice K. C. (1991) Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. *J. Neurosci.* **11,** 563–583.
31. Matsuda L. A., Bonner T. I., and Lolait S. J. (1993) Localization of cannabinoid receptor mRNA in rat brain. *J. Comp. Neurol.* **327,** 535–550.

32. Galiegue S., Mary S., Marchand J., Dussossoy D., Carriere D., Carayon P., et al. (1995) Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. *Eur. J. Biochem.* **232,** 54–61.
33. Jarai Z., Wagner J. A., Varga K., Lake K. D., Compton D. R., Martin B. R., et al. (1999) Cannabinoid-induced mesenteric vasodilation through an endothelial site distinct from CB1 or CB2 receptors. *Proc. Natl. Acad. Sci. USA* **96,** 14, 136–14, 141.
34. Di Marzo, V., Breivogel C. S., Tao Q., Bridgen D. T., Razdan R. K., Zimmer A. M., et al. (2000) Levels, metabolism, and pharmacological activity of anandamide in CB(1) cannabinoid receptor knockout mice: evidence for non-CB(1), non-CB(2) receptor-mediated actions of anandamide in mouse brain. *J. Neurochem.* **75,** 2434–2444.
35. Herkenham M., Lynn A. B., de Costa B. R., and Richfield E. K. (1991) Neuronal localization of cannabinoid receptors in the basal ganglia of the rat. *Brain Res.* **547,** 267–274.
36. Herkenham M., Lynn A. B., Little M. D., Johnson M. R., Melvin L. S., de Costa B. R., and Rice K. C. (1990) Cannabinoid receptor localization in brain. *Proc. Natl. Acad. Sci. USA* **87,** 1932–1936.
37. Mailleux P. and Vanderhaeghen J. J. (1992) Localization of cannabinoid receptor in the human developing and adult basal ganglia. Higher levels in the striatonigral neurons. *Neurosci. Lett.* **148,** 173–176.
38. Mailleux P., Parmentier M., and Vanderhaeghen J. J. (1992) Distribution of cannabinoid receptor messenger RNA in the human brain: an in situ hybridization histochemistry with oligonucleotides. *Neurosci. Lett.* **143,** 200–204.
39. Jansen E. M., Haycock D. A., Ward S. J., and Seybold V. S. (1992) Distribution of cannabinoid receptors in rat brain determined with aminoalkylindoles. *Brain Res.* **575,** 93–102.
40. Thomas B. F., Wei X., and Martin B. R. (1992) Characterization and autoradiographic localization of the cannabinoid binding site in rat brain using [^{3}H] 11-OH-delta 9-THC-DMH. *J. Pharmacol. Exp. Ther.* **263,** 1383–1390.
41. Tsou K., Brown S., Sanudo-Pena M. C., Mackie K., and Walker J. M. (1998) Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. *Neuroscience* **83,** 393–411.
42. Egertova M. and Elphick M. R. (2000) Localisation of cannabinoid receptors in the rat brain using antibodies to the intracellular C-terminal tail of CB. *J. Comp. Neurol.* **422,** 159–171.
43. Moldrich G. and Wenger T. (2000) Localization of the CB(1) cannabinoid receptor in the rat brain. An immunohistochemical study. *Peptides* **21,** 1735–1742.
44. Ishac E. J., Jiang L., Lake K. D., Varga K., Abood M. E., and Kunos G. (1996) Inhibition of exocytotic noradrenaline release by presynaptic cannabinoid CB1 receptors on peripheral sympathetic nerves. *Br. J. Pharmacol.* **118,** 2023–2028.
45. Calignano A., Katona I., Desarnaud F., Giuffrida A., La Rana G., Mackie K., et al. (2000) Bidirectional control of airway responsiveness by endogenous cannabinoids. *Nature* **408,** 96–101.
46. Gerard C. M., Mollereau C., Vassart G., and Parmentier M. (1991) Molecular cloning of a human cannabinoid receptor which is also expressed in testis. *Biochem. J.* **279,** 129–134.
47. Sugiura T., Kodaka T., Nakane S., Kishimoto S., Kondo S., and Waku K. (1998) Detection of an endogenous cannabimimetic molecule, 2-arachidonoylglycerol, and cannabinoid CB1 receptor mRNA in human vascular cells: is 2-arachidonoylglycerol a possible vasomodulator? *Biochem. Biophys. Res. Commun.* **243,** 838–843.
48. Croci T., Manara L., Aureggi G., Guagnini F., Rinaldi-Carmona M., Maffrand J. P., et al. (1998) In vitro functional evidence of neuronal cannabinoid CB1 receptors in human ileum. *Br. J. Pharmacol.* **125,** 1393–1395.
49. Howlett A. C., Qualy J. M., and Khachatrian L. L. (1986) Involvement of Gi in the inhibition of adenylate cyclase by cannabimimetic drugs. *Mol. Pharmacol.* **29,** 307–313.
50. Felder C. C., Joyce K. E., Briley E. M., Mansouri J., Mackie K., Blond O., et al. (1995) Comparison of the pharmacology and signal transduction of the human cannabinoid CB1 and CB2 receptors. *Mol. Pharmacol.* **48,** 443–450.
51. Mackie K., Lai Y., Westenbroek R., and Mitchell R. (1995) Cannabinoids activate an inwardly rectifying potassium conductance and inhibit Q-type calcium currents in AtT20 cells transfected with rat brain cannabinoid receptor. *J. Neurosci.* **15,** 6552–6561.
52. Howlett A. C., Mukhopadhyay S., Shim J. Y., and Welsh W. J. (1999) Signal transduction of

eicosanoid CB1 receptor ligands. *Life Sci.* **65,** 617–625.
53. Glass M. and Felder C. C. (1997) Concurrent stimulation of cannabinoid CB1 and dopamine D2 receptors augments cAMP accumulation in striatal neurons: evidence for a Gs linkage to the CB1 receptor. *J. Neurosci.* **17,** 5327–5333.
54. Maneuf Y. P. and Brotchie J. M. (1997) Paradoxical action of the cannabinoid WIN 55, 212-2 in stimulated and basal cyclic AMP accumulation in rat globus pallidus slices. *Br. J. Pharmacol.* **120,** 1397–1398.
55. Hampson R. E., Mu J., and Deadwyler S. A. (2000) Cannabinoid and kappa opioid receptors reduce potassium K current via activation of G(s) proteins in cultured hippocampal neurons. *J. Neurophysiol.* **84,** 2356–2364.
56. Rubino T., Viganò D., Massi P., Spinello M., Zagato E., Giagnoni G., and Parolaro D. (2000) Chronic delta-9-tetrahydrocannabinol treatment increases cAMP levels and cAMP-dependent protein kinase activity in some rat brain regions. *Neuropharmacology* **39,** 1331–1336.
57. Rhee M. H., Bayewitch M., Avidor-Reiss T., Levy R., and Vogel Z. (1998) Cannabinoid receptor activation differentially regulates the various adenylyl cyclase isozymes. *J. Neurochem.* **71,** 1525–1534.
58. Bouaboula M., Poinot-Chazel C., Bourrie B., Canat X., Calandra B., Rinaldi-Carmona M., et al. (1995) Activation of mitogen-activated protein kinases by stimulation of the central cannabinoid receptor CB1. *Biochem. J.* **312,** 637–641.
59. Rueda D., Galve-Roperh I., Haro A., and Guzman M. (2000) The CB(1) cannabinoid receptor is coupled to the activation of c-Jun N-terminal kinase. *Mol. Pharmacol.* **58,** 814–820.
60. Liu J., Gao B., Mirshahi F., Sanyal A. J., Khanolkar A. D., Makriyannis A., and Kunos G. (2000) Functional CB1 cannabinoid receptors in human vascular endothelial cells. *Biochem. J.* **346,** 835–840.
61. Sanchez C., Galve-Roperh I., Rueda D., and Guzman M. (1998) Involvement of sphingomyelin hydrolysis and the mitogen-activated protein kinase cascade in the Delta9-tetrahydrocannabinol-induced stimulation of glucose metabolism in primary astrocytes. *Mol. Pharmacol.* **54,** 834–843.
62. Gomez D. P., Velasco G., and Guzman M. (2000) The CB1 cannabinoid receptor is coupled to the activation of protein kinase B/Akt. *Biochem. J.* **347,** 369–373.
63. Bouaboula M., Bourrie B., Rinaldi-Carmona M., Shire D., Le Fur G., and Casellas P. (1995) Stimulation of cannabinoid receptor CB1 induces krox-24 expression in human astrocytoma cells. *J. Biol. Chem.* **270,** 13,973–13,980.
64. Derkinderen P., Toutant M., Burgaya F., Le Bert M., Siciliano J. C., de Franciscis V., et al. (1996) Regulation of a neuronal form of focal adhesion kinase by anandamide. *Science* **273,** 1719–1722.
65. Shivachar A. C., Martin B. R., and Ellis E. F. (1996) Anandamide- and delta9-tetrahydrocannabinol-evoked arachidonic acid mobilization and blockade by SR141716A [N-(Piperidin-1-yl)-5-(4-chlorophenyl)-1-(2, 4-dichlorophenyl)-4 -methyl-1H-pyrazole-3-carboximide hydrochloride]. *Biochem. Pharmacol.* **51,** 669–676.
66. Vasquez C. and Lewis D. L. (1999) The CB1 cannabinoid receptor can sequester G-Proteins, making them unavailable to couple to other receptors. *J. Neurosci.* **19,** 9271–9280.
67. Prather P. L., Martin N. A., Breivogel C. S., and Childers S. R. (2000) Activation of cannabinoid receptors in rat brain by WIN 55212-2 produces coupling to multiple G protein alpha-subunits with different potencies. *Mol. Pharmacol.* **57,** 1000–1010.
68. Mackie K. and Hille B. (1992) Cannabinoids inhibit N-type calcium channels in neuroblastoma-glioma cells. *Proc. Natl. Acad. Sci. USA* **89,** 3825–3829.
69. Pan X., Ikeda S. R., and Lewis D. L. (1996) Rat brain cannabinoid receptor modulates N-type Ca^{2+} channels in a neuronal expression system. *Mol. Pharmacol.* **49,** 707–714.
70. Gebremedhin D., Lange A. R., Campbell W. B., Hillard C. J., and Harder D. R. (1999) Cannabinoid CB1 receptor of cat cerebral arterial muscle functions to inhibit L-type Ca^{2+} channel current. *Am. J. Physiol.* **276,** H2085–H2093.
71. McAllister S. D., Griffin G., Satin L. S., and Abood M. E. (1999) Cannabinoid receptors can activate and inhibit G protein-coupled inwardly rectifying potassium channels in a Xenopus oocyte expression system. *J. Pharmacol. Exp. Ther.* **291,** 618–626.
72. Deadwyler S. A., Hampson R. E., Bennett B. A., Edwards T. A., Mu J., Pacheco M. A., et al. (1993) Cannabinoids modulate potassium current in cultured hippocampal neurons. *Receptors. Channels* **1,** 121–134.
73. Deadwyler S. A., Hampson R. E., Mu J., Whyte A., and Childers S. (1995) Cannabinoids mod-

ulate voltage sensitive potassium A-current in hippocampal neurons via a cAMP-dependent process. *J. Pharmacol. Exp. Ther.* **273,** 734–743.
74. Mu J., Zhuang S. Y., Hampson R. E., and Deadwyler S. A. (2000) Protein kinase-dependent phosphorylation and cannabinoid receptor modulation of potassium A current (IA) in cultured rat hippocampal neurons. *Pflugers Arch.* **439,** 541–546.
75. Schweitzer P. (2000) Cannabinoids decrease the K(+) M-current in hippocampal CA1 neurons. *J. Neurosci.* **20,** 51–58.
76. Szabo B., Dorner L., Pfreundtner C., Norenberg W., and Starke K. (1998) Inhibition of GABAergic inhibitory postsynaptic currents by cannabinoids in rat corpus striatum. *Neuroscience* **85,** 395–403.
77. Katona I., Sperlagh B., Sik A., Kafalvi A., Vizi E. S., Mackie K., and Freund T. F. (1999) Presynaptically located CB1 cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons. *J. Neurosci.* **19,** 4544–4558.
78. Vaughan C. W., McGregor I. S., and Christie M. J. (1999) Cannabinoid receptor activation inhibits GABAergic neurotransmission in rostral ventromedial medulla neurons in vitro. *Br. J. Pharmacol.* **127,** 935–940.
79. Hajos N., Katona I., Naiem S. S., Mackie K., Ledent C., Mody I., and Freund T. F. (2000) Cannabinoids inhibit hippocampal GABAergic transmission and network oscillations. *Eur. J. Neurosci.* **12,** 3239–3249.
80. Hoffman A. F. and Lupica C. R. (2000) Mechanisms of cannabinoid inhibition of GABA(A) synaptic transmission in the hippocampus. *J. Neurosci.* **20,** 2470–2479.
81. Takahashi K. A. and Linden D. J. (2000) Cannabinoid receptor modulation of synapses received by cerebellar Purkinje cells. *J. Neurophysiol.* **83,** 1167–1180.
82. Waksman Y., Olson J. M., Carlisle S. J., and Cabral G. A. (1999) The central cannabinoid receptor (CB1) mediates inhibition of nitric oxide production by rat microglial cells. *J. Pharmacol. Exp. Ther.* **288,** 1357–1366.
83. Molina-Holgado F., Lledo A., and Guaza C. (1997) Anandamide suppresses nitric oxide and TNF-alpha responses to Theiler's virus or endotoxin in astrocytes. *Neuroreport* **8,** 1929–1933.
84. Hillard C. J., Muthian S., and Kearn C. S. (1999) Effects of CB(1) cannabinoid receptor activation on cerebellar granule cell nitric oxide synthase activity. *FEBS Lett.* **459,** 277–281.
85. Coffey R. G., Yamamoto Y., Snella E., and Pross S. (1996) Tetrahydrocannabinol inhibition of macrophage nitric oxide production. *Biochem. Pharmacol.* **52,** 743–751.
86. Cadogan A. K., Alexander S. P., Boyd E. A., and Kendall D. A. (1997) Influence of cannabinoids on electrically evoked dopamine release and cyclic AMP generation in the rat striatum. *J. Neurochem.* **69,** 1131–1137.
87. Gessa G. L., Melis M., Muntoni A. L., and Diana M. (1998) Cannabinoids activate mesolimbic dopamine neurons by an action on cannabinoid CB1 receptors. *Eur. J. Pharmacol.* **341,** 39–44.
88. Carta G., Nava F., and Gessa G. L. (1998) Inhibition of hippocampal acetylcholine release after acute and repeated Delta9-tetrahydrocannabinol in rats. *Brain Res.* **809,** 1–4.
89. Gessa G. L., Casu M. A., Carta G., and Mascia M. S. (1998) Cannabinoids decrease acetylcholine release in the medial-prefrontal cortex and hippocampus, reversal by SR 141716A. *Eur. J. Pharmacol.* **355,** 119–124.
90. Kathmann M., Weber B., and Schlicker E. (2001) Cannabinoid CB1 receptor-mediated inhibition of acetylcholine release in the brain of NMRI, CD-1 and C57BL/6J mice. Naunyn Schmiedebergs *Arch. Pharmacol.* **363,** 50–56.
91. Gifford A. N., Bruneus M., Gatley S. J., and Volkow N. D. (2000) Cannabinoid receptor-mediated inhibition of acetylcholine release from hippocampal and cortical synaptosomes. *Br. J. Pharmacol.* **131,** 645–650.
92. Nakazi M., Bauer U., Nickel T., Kathmann M., and Schlicker E. (2000) Inhibition of serotonin release in the mouse brain via presynaptic cannabinoid CB1 receptors. *Naunyn Schmiedebergs Arch. Pharmacol.* **361,** 19–24.
93. Skaper S. D., Buriani A., Dal Toso R., Petrelli L., Romanello S., Facci L., and Leon A. (1996) The ALIAmide palmitoylethanolamide and cannabinoids, but not anandamide, are protective in a delayed postglutamate paradigm of excitotoxic death in cerebellar granule neurons. *Proc. Natl. Acad. Sci. USA* **93,** 3984–3989.
94. Ross R. A., Coutts A. A., McFarlane S. M., Anavi-Goffer S., Irving A. J., Pertwee R. G., et al. (2001) Actions of cannabinoid receptor ligands on rat cultured sensory neurones: implications for antinociception. *Neuropharmacology* **40,** 221–232.

95. Kearn C. S. and Hillard C. J. (1997) Rat microglial cells express the peripheral type cannabinoid receptor (CB2) which is negatively coupled to adenylate cyclase. (Abstract) ICRS June 20–22, 1997, Stone Mountain, Ga, `USA.
96. Bayewitch M., Avidor-Reiss T., Levy R., Barg J., Mechoulam R., and Vogel Z. (1995) The peripheral cannabinoid receptor: adenylate cyclase inhibition and G protein coupling. *FEBS Lett.* **375,** 143–147.
97. Condie R., Herring A., Koh W. S., Lee M., and Kaminski N. E. (1996) Cannabinoid inhibition of adenylate cyclase-mediated signal transduction and interleukin 2 (IL-2) expression in the murine T-cell line, EL4.IL-2. *J. Biol. Chem.* **271,** 13,175–13,183.
98. Jeon Y. J., Yang K. H., Pulaski J. T., and Kaminski N. E. (1996) Attenuation of inducible nitric oxide synthase gene expression by delta 9-tetrahydrocannabinol is mediated through the inhibition of nuclear factor- kappa B/Rel activation. *Mol. Pharmacol.* **50,** 334–341.
99. Schatz A. R., Lee M., Condie R. B., Pulaski J. T., and Kaminski N. E. (1997) Cannabinoid receptors CB1 and CB2: a characterization of expression and adenylate cyclase modulation within the immune system. *Toxicol. Appl. Pharmacol.* **142,** 278–287.
100. Bouaboula M., Poinot-Chazel C., Marchand J., Canat X., Bourrie B., Rinaldi-Carmona M., et al. (1996) Signaling pathway associated with stimulation of CB2 peripheral cannabinoid receptor. Involvement of both mitogen-activated protein kinase and induction of Krox-24 expression. *Eur. J. Biochem.* **237,** 704–711.
101. Herring A. C., Koh W. S., and Kaminski N. E. (1998) Inhibition of the cyclic AMP signaling cascade and nuclear factor binding to CRE and kappaB elements by cannabinol, a minimally CNS-active cannabinoid. *Biochem. Pharmacol.* **55,** 1013–1023.
102. Derocq J. M., Jbilo O., Bouaboula M., Segui M., Clere C., and Casellas P. (2000) Genomic and functional changes induced by the activation of the peripheral cannabinoid receptor CB2 in the promyelocytic cells HL-60. Possible involvement of the CB2 receptor in cell differentiation. *J. Biol. Chem.* **275,** 15,621–15,628.
103. Ouyang Y., Hwang S. G., Han S. H., and Kaminski N. E. (1998) Suppression of interleukin-2 by the putative endogenous cannabinoid 2-arachidonyl-glycerol is mediated through down-regulation of the nuclear factor of activated T cells. *Mol. Pharmacol.* **53,** 676–683.
104. Klein T. W., Newton C., and Friedman H. (1998) Cannabinoid receptors and immunity. *Immunol. Today* **19,** 373–381.
105. Patrini G., Sacerdote P., Fuzio D., Manfredi B., and Parolaro D. (1997) Regulation of immune functions in rat splenocytes after acute and chronic in vivo treatment with CP-55,940, a synthetic cannabinoid compound. *J. Neuroimmunol.* **80,** 143–148.
106. Cabral G. A. and Dove Pettit D. (1998) Drugs and immunity: cannabinoids and their role in decreased resistance to infectious disease. *J. Neuroimmunol.* **83,** 116–123.
107. Massi P., Sacerdote P., Ponti W., Fuzio D., Manfredi B., Vigano D., et al. (1998) Immune function alterations in mice tolerant to delta9-tetrahydrocannabinol: functional and biochemical parameters. *J. Neuroimmunol.* **92,** 60–66.
108. Kaminski N. E. (1998) Regulation of the cAMP cascade, gene expression and immune function by cannabinoid receptors. *J. Neuroimmunol.* **83,** 124–132.
109. Buckley N. E., McCoy K. L., Mezey E., Bonner T., Zimmer A., Felder C. C., and Glass M. (2000) Immunomodulation by cannabinoids is absent in mice deficient for the cannabinoid CB(2) receptor. *Eur. J. Pharmacol.* **396,** 141–149.
110. Fischer-Stenger K., Dove Pettit D., and Cabral G. A. (1993) Delta 9-tetrahydrocannabinol inhibition of tumor necrosis factor-alpha: suppression of post-translational events. *J. Pharmacol. Exp. Ther.* **267,** 1558–1565.
111. Smith S. R., Terminelli C., and Denhardt G. (2000) Effects of cannabinoid receptor agonist and antagonist ligands on production of inflammatory cytokines and anti-inflammatory interleukin-10 in endotoxemic mice. *J. Pharmacol. Exp. Ther.* **293,** 136–150.
112. Puffenbarger R. A., Boothe A. C., and Cabral G. A. (2000) Cannabinoids inhibit LPS-inducible cytokine mRNA expression in rat microglial cells. *Glia* **29,** 58–69.
113. Zhu W., Newton C., Daaka Y., Friedman H., and Klein T. W. (1994) Delta9-tetrahydrocannabinol enhances the secretion of interleukin-1 from endotoxin-stimulated macrophages. *J. Pharmacol. Exp. Ther.* **270,** 1334–1339.
114. Klein T. W., Newton C., Widen R., and Friedman H. (1993) Delta 9-tetrahydrocannabinol injection induces cytokine-mediated mortality

of mice infected with *Legionella pneumophila. J. Pharmacol. Exp. Ther.* **267,** 635–640.
115. Newton C., Klein T., and Friedman H. (1998) The role of macrophages in THC-induced alteration of the cytokine network. *Adv. Exp. Med. Biol.* **437,** 207–214.
116. Jbilo O., Derocq J. M., Segui M., Le Fur G., and Casellas P. (1999) Stimulation of peripheral cannabinoid receptor CB2 induces MCP-1 and IL-8 gene expression in human promyelocytic cell line HL60. *FEBS Lett.* **448,** 273–277.
117. Baggiolini M. and Clark-Lewis I. (1992) Interleukin-8, a chemotactic and inflammatory cytokine. *FEBS Lett.* **307,** 97–101.
118. Valente A. J., Graves D. T., Vialle-Valentin C. E., Delgado R., and Schwartz C. J. (1988) Purification of a monocyte chemotactic factor secreted by non human primate vascular cells in culture. *Biochemistry* **27,** 4162–4168.
119. Sacerdote P., Massi P., Panerai A. E., and Parolaro D. (2000) *In vivo* and *in vitro* treatment with the synthetic cannabinoid CP55940 decreases the *in vitro* migration of macrophages in the rat: involvement of both CB1 and CB2 receptors. *J. Neuroimmunol.* **109,** 155–163.
120. Howlett A. C., Champion T. M., Wilken G. H., and Mechoulam R. (1990) Stereochemical effects of 11-OH-delta 8-tetrahydrocannabinol-dimethylheptyl to inhibit adenylate cyclase and bind to the cannabinoid receptor. *Neuropharmacology* **29,** 161–165.
121. Mechoulam R., Feigenbaum J. J., Lander N., Segal M., Jarbe T. U., Hiltunen A. J., and Consroe P. (1988) Enantiomeric cannabinoids: stereospecificity of psychotropic activity. *Experientia* **44,** 762–764.
122. Feigenbaum J. J., Bergmann F., Richmond S. A., Mechoulam R., Nadler V., Kloog Y., and Sokolovsky M. (1989) Non-psychotropic cannabinoid acts as a functional N-methyl-D-aspartate receptor blocker. *Proc. Natl. Acad. Sci. USA* **86,** 9584–9587.
123. Nadler V., Mechoulam R., and Sokolovsky M. (1993) The non-psychotropic cannabinoid (+)-(3S,4S)-7-hydroxy-delta 6- tetrahydrocannabinol 1,1-dimethylheptyl (HU-211) attenuates N-methyl-D-aspartate receptor-mediated neurotoxicity in primary cultures of rat forebrain. *Neurosci. Lett.* **162,** 43–45.
124. Nadler V., Biegon A., Beit-Yannai E., Adamchik J., and Shohami E. (1995) ^{45}Ca accumulation in rat brain after closed head injury; attenuation by the novel neuroprotective agent HU-211. *Brain Res.* **685,** 1–11.
125. Eshhar N., Striem S., Kohen R., Tirosh O., and Biegon A. (1995) Neuroprotective and antioxidant activities of HU-211, a novel NMDA receptor antagonist. *Eur. J. Pharmacol.* **283,** 19–29.
126. Hampson A. J., Bornheim L. M., Scanziani M., Yost C. S., Gray A. T., Hansen B. M., et al. (1998) Dual effects of anandamide on NMDA receptor-mediated responses and neurotransmission. *J. Neurochem.* **70,** 671–676.
127. Di Marzo, V., Bisogno T., Melck D., Ross R., Brockie H., Stevenson L., et al. (1998) Interactions between synthetic vanilloids and the endogenous cannabinoid system. *FEBS Lett.* **436,** 449–454.
128. Beltramo M. and Piomelli D. (1999) Anandamide transport inhibition by the vanilloid agonist olvanil. *Eur. J. Pharmacol.* **364,** 75–78.
129. Zygmunt P. M., Petersson J., Andersson D. A., Chuang H., Sorgard M., Di Marzo V., et al. (1999) Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. *Nature* **400,** 452–457.
130. Smart D., Gunthorpe M. J., Jerman J. C., Nasir S., Gray J., Muir A. I., et al. (2000) The endogenous lipid anandamide is a full agonist at the human vanilloid receptor (hVR1). *Br. J. Pharmacol.* **129,** 227–230.
131. Smart D. and Jerman J. C. (2000) Anandamide: an endogenous activator of the vanilloid. *Trends Pharmacol. Sci.* **134** 21, 134
132. Szolcsanyi J. (2000) Anandamide and the question of its functional role for activation of capsaicin receptors. *Trends Pharmacol. Sci.* **21,** 203–204.
133. Szolcsanyi J. (2000) Are cannabinoids endogenous ligands for the VR1 capsaicin receptor?. *Trends Pharmacol. Sci.* **21,** 41–42.
134. Zygmunt P. M., Julius I., Di Marzo V., and Hogestatt E. D. (2000) Anandamide: the other side of the coin. *Trends Pharmacol. Sci.* **21,** 43–44.
135. Maccarrone M., Lorenzon T., Bari M., Melino G., and Finazzi-Agro A. (2000) Anandamide induces apoptosis in human cells via vanilloid receptors. Evidence for a protective role of cannabinoid receptor. *J. Biol. Chem.* **275,** 31,938–31,945.
136. Lagalwar S., Bordayo E. Z., Hoffmann K. L., Fawcett J. R., and Frey W. H. (1999) Anandamide inhibit binding to the muscarinic acetylcholine receptor. *J. Mol. Neurosci.* **13,** 55–61.

137. Kimura T., Ohta T., Watanabe K., Yoshimura H., and Yamamoto I. (1998) Anandamide, an endogenous cannabinoid receptor ligand, also interacts with 5-hydroxytryptamine (5-HT) receptor. *Bio. Pharm. Bull.* **21,** 224–226.
138. Venance L., Piomelli D., Glowinski J., and Giaume C. (1995) Inhibition by anandamide of gap junctions and intercellular calcium signalling in striatal astrocytes. *Nature* **376,** 590–594.
139. Sagan S., Venance L., Torrens Y., Cordier J., Glowinski J., and Giaume C. (1999) Anandamide and WIN 55212-2 inhibit cyclic AMP formation through G-protein-coupled receptors distinct from CB1 cannabinoid receptors in cultured astrocytes. *Eur. J. Neurosci.* **11,** 691–699.
140. Rodriguez J. J., Mackie K., and Pickel V. M. (2001) Ultrastructural localisation of the CB1 cannabinoid receptor in μ-opiod receptor patches of the rat caudate putamen necleus, *J. Neurosci.* **21,** 823–833.
141. Budd S. L., and Lipton S. A. (1998) Calcium tsunamis: do astrocytes transmit cell death messages via gap junctions during ischemia? *Nat. Neurosci.* **1,** 431–432.
142. Lin J. H., Weigel H., Cotrina M. L., Liu S., Bueno E., Hansen A. J., et al. (1998) Gap-junction-mediated propagation and amplification of cell injury. *Nat. Neurosci.* **1,** 494–500.
143. Martinez A. D., and Saez J. C. (2000) Regulation of astrocyte gap junctions by hypoxia-reoxygenation. *Brain Res. Brain Res. Rev.* **32,** 250–258.
144. Bains J. S., and Shaw C. A. (1997) Neurodegenerative disorders in humans: the role of glutathione in oxidative stress-mediated neuronal death. *Brain Res. Brain Res. Rev.* **25,** 335–358.
145. Hensley K., Pye Q. N., Tabatabaie T., Stewart C. A., and Floyd R. A. (1998) Reactive oxygen involvement in neurodegenerative pathways in *Neuroinflammation: Mechanisms and Management* (Wood P. L., ed.), Humana Press, Totowa, NJ, p.265–281.
146. Hampson A. J., Grimaldi M., Axelrod J., and Wink D. (1998) Cannabidiol and (–)Delta9-tetrahydrocannabinol are neuroprotective antioxidants. *Proc. Natl. Acad. Sci. USA* **95,** 8268–8273.
147. Gulaya N. M., Kuzmenko A. I., Margitich V. M., Govseeva N. M., Melnichuk S. D., Goridko T. M., and Zhukov A. D. (1998) Long-chain N-acylethanolamines inhibit lipid peroxidation in rat liver mitochondria under acute hypoxic hypoxia. *Chem. Phys. Lipids* **97,** 49–54.
148. Calignano A., La Rana G., Giuffrida A., and Piomelli D. (1998) Control of pain initiation by endogenous cannabinoids. *Nature* **394,** 277–281.
149. Thomas B. F., Compton D. R., and Martin B. R. (1990) Characterization of the lipophilicity of natural and synthetic analogs of delta 9-tetrahydrocannabinol and its relationship to pharmacological potency. *J. Pharmacol. Exp. Ther.* **255,** 624–630.
150. Doble A. (1999) The role of excitotoxicity in neurodegenerative disease: implications for therapy. *Pharmacol. ther.* **81,** 163–221.
151. Auclair N., Otani S., Soubrie P., and Crepel F. (2000) Cannabinoids modulate synaptic strength and plasticity at glutamatergic synapses of rat prefrontal cortex pyramidal neurons. *J. Neurophysiol.* **83,** 3287–3293.
152. Szabo B., Wallmichrath I., Mathonia P., and Pfreundtner C. (2000) Cannabinoids inhibit exacitatory neurotransmission in the substantia nigra pars reticulata. *Neuroscience* **97,** 89–97.
153. Vaughan C. W., Connor M., Bagley E. E., and Christie M. J. (2000) Actions of cannabinoids on membrane properties and synaptic transmission in rat periaqueductal gray neurons in vitro. *Mol. Pharmacol.* **57,** 288–295.
154. Levenes C., Daniel H., Soubrie P., and Crepel F. (1998) Cannabinoids decrease excitatory synaptic transmission and impair long-term depression in rat cerebellar Purkinje cells. *J. Physiol.* (Lond) **510,** 867–879.
155. Ameri A., Wilhelm A., and Simmet T. (1999) Effects of the endogeneous cannabinoid, anandamide, on neuronal activity in rat hippocampal slices. *Br. J. Pharmacol.* **126,** 1831–1839.
156. Shen M. and Thayer S. A. (1999) Delta9-tetrahydrocannabinol acts as a partial agonist to modulate glutamatergic synaptic transmission between rat hippocampal neurons in culture. *Mol. Pharmacol.* **55,** 8–13.
157. Kim D. J. and Thayer S. A. (2000) Activation of CB1 cannabinoid receptors inhibits neurotransmitter release from identified synaptic sites in rat hippocampal cultures. *Brain Res.* **852,** 398–405.
158. Gerdeman G. and Lovinger D. M. (2001) CB1 Cannabinoid receptor inhibits synaptic release of glutamate in rat dorsolateral striatum. *J. Neurophysiol.* **85,** 468–471.
159. Eshhar N., Striem S., and Biegon A. (1993) HU-211, a non-psychotropic cannabinoid, rescues

cortical neurones from excitatory amino acid toxicity in culture. *Neuroreport* **5,** 237–240.
160. Shen M. and Thayer S. A. (1998) Cannabinoid receptor agonists protect cultured rat hippocampal neurons from excitotoxicity. *Mol. Pharmacol.* **54,** 459–462.
161. Nagayama T., Sinor A. D., Simon R. P., Chen J., Graham S. H., Jin K., and Greenberg D. A. (1999) Cannabinoids and neuroprotection in global and focal cerebral ischemia and in neuronal cultures. *J. Neurosci.* **19,** 2987–2995.
162. Sinor A. D., Irvin S. M., and Greenberg D. A. (2000) Endocannabinoids protect cerebral cortical neurons from in vitro ischemia in rats. *Neurosci. Lett.* **278,** 157–160.
163. Vered M., Bar-Joseph A., Belayev L., Berkovich Y., and Biegon A. (1994) Anti-ischemia activity of HU-211, a non-psychotropic synthetic cannabinoid. *Acta Neurochir. Suppl. (Wien)* **60,** 335–337.
164. Bar-Joseph A., Berkovitch Y., Adamchik J., and Biegon A. (1994) Neuroprotective activity of HU-211, a novel NMDA antagonist, in global ischemia in gerbils. *Mol. Chem. Neuropathol.* **23,** 125–135.
165. Belayev L., Bar-Joseph A., Adamchik J., and Biegon A. (1995) HU-211, a non-psychotropic cannabinoid, improves neurological signs and reduces brain damage after severe forebrain ischemia in rats. *Mol. Chem. Neuropathol.* **25,** 19–33.
166. Belayev L., Busto R., Zhao W., and Ginsberg M. D. (1995) HU-211, a novel non-competitive N-methyl-D-aspartate antagonist, improves neurological deficit and reduces infarct volume after reversible focal cerebral ischemia in the rat. *Stroke* **26,** 2313–2319.
167. Belayev L., Busto R., Watson B. D., and Ginsberg M. D. (1995) Post-ischemic administration of HU-211, a novel non-competitive NMDA antagonist, protects against blood-brain barrier disruption in photochemical cortical infarction in rats: a quantitative study. *Brain Res.* **702,** 266–270.
168. Leker R. R., Shohami E., Abramsky O., and Ovadia H. (1999) Dexanabinol; a novel neuroprotective drug in experimental focal cerebral ischemia. *J. Neurol. Sci.* **162,** 114–119.
169. Louw D. F., Yang F. W., and Sutherland G. R. (2000) The effect of delta-9-tetrahydrocannabinol on forebrain ischemia in rat. *Brain Res.* **857,** 183–187.
170. Lake K. D., Compton D. R., Varga K., Martin B. R., and Kunos G. (1997) Cannabinoid-induced hypotension and bradycardia in rats mediated by CB1-like cannabinoid receptors. *J. Pharmacol. Exp. Ther.* **281,** 1030–1037.
171. Zimmer A., Zimmer A. M., Hohmann A. G., Herkenham M., and Bonner T. I. (1999) Increased mortality, hypoactivity, and hypoalgesia in cannabinoid CB1 receptor knockout mice. *Proc. Natl. Acad. Sci. USA* **96,** 5780–5785.
172. Shohami E., Novikov M., and Mechoulam R. (1993) A non-psychotropic cannabinoid, HU-211, has cerebroprotective effects after closed head injury in the rat. *J. Neurotrauma* **10,** 109–119.
173. Shohami E., Gallily R., Mechoulam R., Bass R., and Ben-Hur T. (1997) Cytokine production in the brain following closed head injury: dexanabinol (HU-211) is a novel TNF-alpha inhibitor and an effective neuroprotectant. *J. Neuroimmunol.* **72,** 169–177.
174. Brewster M. E., Pop E., Foltz R. L., Reuschel S., Griffith W., Amselem S., and Biegon A. (1997) Clinical pharmacokinetics of escalating i.v. doses of dexanabinol (HU-211), a neuroprotectant agent, in normal volunteers. *Int. J. Clin. Pharmacol. Ther.* **35,** 361–365.
175. Molina-Holgado F., Molina-Holgado E., and Guaza C. (1998) The endogenous cannabinoid anandamide potentiates interleukin-6 production by astrocytes infected with Theiler's murine encephalomyelitis virus by a receptor-mediated pathway. *FEBS Lett.* **433,** 139–142.
176. Racke M. K., Ratts R. B., Arredondo L., Perrin P. J., and Lovett-Racke A. (2000) The role of costimulation in autoimmune demyelination. *J. Neuroimmunol.* **107,** 205–215.
177. Lyman W. D., Sonett J. R., Brosnan C. F., Elkin R., and Bornstein M. B. (1989) Delta 9-tetrahydrocannabinol: a novel treatment for experimental autoimmune encephalomyelitis. *J. Neuroimmunol.* **23,** 73–81.
178. Wirguin I., Mechoulam R., Breuer A., Schezen E., Weidenfeld J., and Brenner T. (1994) Suppression of experimental autoimmune encephalomyelitis by cannabinoids. *Immunopharmacology* **28,** 209–214.
179. Achiron A., Miron S., Lavie V., Margalit R., and Biegon A. (2000) Dexanabinol (HU-211) effect on experimental autoimmune encephalomyelitis: implications for the treatment of acute relapses of multiple sclerosis. *J. Neuroimmunol.* **102,** 26–31.

180. Baker D., Pryce G., Croxford J. L., Brown P., Pertwee R. G., Huffman J. W., and Layward L. (2000) Cannabinoids control spasticity and tremor in a multiple sclerosis model. *Nature* **404,** 84–87.
181. Baker D., Pryce G., Croxford J. L., Brown P., Pertwee R. G., Makriyannis A., et al. (2001) Endocannabinoids control spasticity in a multiple sclerosis model. *FASEB J.* **15,** 300–302.
182. Iversen L. L. (2000) *The Science of Marijuana.* Oxford University Press, Oxford, UK.
183. Petro D. J. and Ellenberger C. J. (1981) Treatment of human spasticity with delta 9-tetrahydrocannabinol. *J. Clin. Pharmacol.* **21,** 413S–416S.
184. Clifford D. B. (1983) Tetrahydrocannabinol for tremor in multiple sclerosis. *Ann. Neurol.* **13,** 669–671.
185. Martyn C. N., Illis L. S., and Thom J. (1995) Nabilone in the treatment of multiple sclerosis. *Lancet* **345,** 579.
186. Consroe P., Musty R., Rein J., Tillery W., and Pertwee R. (1997) The perceived effects of smoked cannabis on patients with multiple sclerosis. *Eur. Neurol.* **38,** 44–48.
187. Sullivan J. M. (2000) Cellular and molecular mechanisms underlying learning and memory impairments produced by cannabinoids. *Learn. Mem.* **7,** 132–139.
188. Mailleux P. and Vanderhaeghen J. J. (1993) Dopaminergic regulation of cannabinoid receptor mRNA levels in the rat caudate-putamen: an in situ hybridization study. *J. Neurochem.* **61,** 1705–1712.
189. Zeng B. Y., Dass B., Owen A., Rose S., Cannizzaro C., Tel B. C., and Jenner P. (1999) Chronic L-DOPA treatment increases striatal cannabinoid CB1 receptor mRNA expression in 6-hydroxydopamine-lesioned rats. *Neurosci. Lett.* **276,** 71–74.
190. Romero J., Berrendero F., Perez-Rosado A., Manzanares J., Rojo A., Fernandez-Ruiz J. J., et al. (2000) Unilateral 6-hydroxydopamine lesions of nigrostriatal dopaminergic neurons increased CB1 receptor mRNA levels in the caudate-putamen. *Life Sci.* **66,** 485–494.
191. Brotchie J. M. (1998) Adjuncts to dopamine replacement: a pragmatic approach to reducing the problem of dyskinesia in Parkinson's disease. *Mov. Disord.* **13,** 871–876.
192. Frankel J. P., Hughes A., Lees A. J., and Stern G. M. (1990) Marijuana for Parkinsonian tremor. *J. Neurol. Neurosurg. Psych.* **53,** 436.
193. Chan G. C., Hinds T. R., Impey S., and Storm D. R. (1998) Hippocampal neurotoxicity of Delta9-tetrahydrocannabinol. *J. Neurosci.* **18,** 5322–5332.
194. Sarker K. P., Obara S., Nakata M., Kitajima I., and Maruyama I. (2000) Anandamide induces apoptosis of PC-12 cells: involvement of superoxide and caspase-3. *FEBS Lett.* **472,** 39–44.