Università di Pisa

Facoltà di Farmacia

Corso di Laurea Specialistica in Chimica e Tecnologia Farmaceutiche

Tesi di Laurea:

Design and synthesis of cannabinoid receptor ligands as potential soft drugs or CB2 selective ligands.

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Alla mia famiglia….

Introduction

Description

Cannabis is a genus of flowering plant that includes one or more species. The plant is believed to be indigenous to Central Asia, China, and the north-west Himalayas. The common name for *Cannabis* is hemp, although this term is sometimes used to refer only to strains cultivated for "industrial" (non-drug) use. *Cannabis* plants produce a unique family of compounds called cannabinoids, several of which produce psychical and/or physiological effects when consumed. The crude drug usually comes in the form of dried flowers and leaves, resin (hashish), or various extracts. The cultivation or possession of *Cannabis* for drug purposes is outlawed in most countries.

Figure 1

Cannabis is an annual, flowering herb. The leaves are palmately compound, with serrate leaflets. The first pair of leaves usually have a single leaflet, the number gradually increasing up to a maximum of about thirteen leaflets per leaf (usually seven or nine), depending on variety and growing conditions.

At the top of a flowering plant, this number again diminishes to a single leaflet per leaf. The lower leaf pairs usually occur in an opposite leaf arrangement and the upper leaf pairs in an alternate arrangement on the main stem of a mature plant.

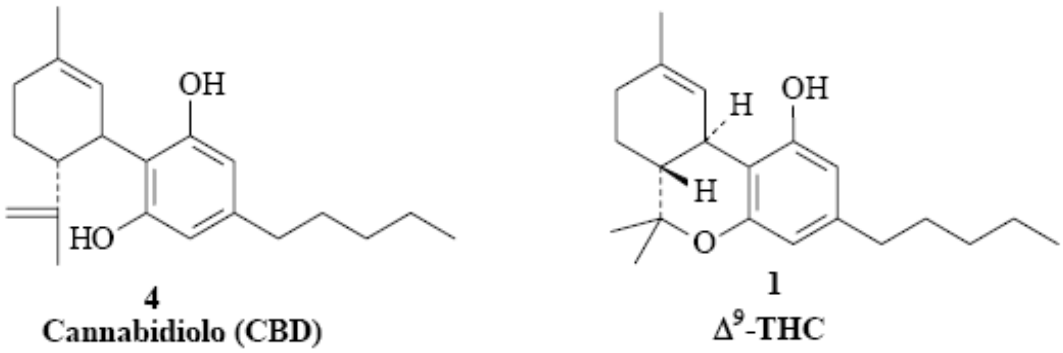
Figure 2. Leaf of a *Cannabis* plant

Taxonomy

The genus *Cannabis* was formerly placed in the Nettle (Urticaceae) or Mulberry (Moraceae) family, but is now considered along with hops (*Humulus* sp.) to belong to the Hemp family (Cannabaceae). Various types of *Cannabis* have been described, and classified as species, subspecies, or varieties:

- plants cultivated for fiber and seed production, described as low-intoxicant, non-drug, o fiber types;
- plants cultivated for drug production, described as high-intoxicant or drug types;
- escaped or wild forms of either of the above types.

Cannabis plants produce a unique family of terpeno-phenolic compounds called cannabinoids, which produce the "high" one experiences from smoking marijuana. The two cannabinoids usually produced in greatest abundance are cannabidiol (CBD) and/or Δ^9-tetrahydrocannabinol (THC), but only THC is psychoactive.



4
Cannabidiolo (CBD)

1
Δ^9-THC

Figure 3

Since the early 1970's, *Cannabis* plants have been categorized by their chemical phenotype or "chemotype," based on the overall amount of THC produced, and on the ratio of THC to CBD. Although overall cannabinoid production is influenced by environmental factors, the THC/CBD ratio is genetically determined and remains fixed throughout the life of a plant. Non-drug plants produce relatively low levels of THC and high levels of CBD, while drug plants produce high levels of THC and low levels of CBD. When plants of these two chemotypes cross-pollinate, the plants in the first filial (F1) generation have an intermediate chemotype and produce similar amounts of CBD and THC. Female plants of this chemotype may produce enough THC to be utilized for drug production.

Figure 4. Top of *Cannabis* plant in vegetative growth stage

Popular usage

The National Institute of Drug Abuse defines drug abuse as the non-medical use of a substance for psychic effect, dependence, or suicide attempt.

Marijuana (also known as ganja) and hashish are psychoactive products of the plant *Cannabis sativa* L. subsp. *indica* (*C. indica* Lam.). The herbal form of the drug consists of dried mature inflorescences and subtending leaves of pistillate ("female") plants. The resinous form consists primarily of glandular trichomes collected from the same plant material. It has been reported that commercial hashish is often no more potent than high quality seedless marijuana.[1]

However, carefully produced and screened hashish is up to three times as potent as the highest quality herb.[2] The major biologically active chemical compound in *Cannabis* is THC. It has psychoactive and physiological effects when consumed, usually by smoking or ingestion. The minimum amount of THC required to have a perceptible psychoactive effect is about 5 mg. A related compound, Δ^9-tetrahydrocannabidivarin, also know as THCV, is produced in appreciable amounts by certain drug strains. This cannabinoid has been described in the popular literature as having shorter-acting, flashier effects than THC, but recent studies suggest that it may actually inhibit the effects of THC. Relatively high levels of THCV are common in African dagga (marijuana), and in hashish from the northwest Himalaya.

The nature and intensity of the immediate effects of cannabis consumption vary according to the dose, the species or hybridization of the source plant, the method of consumption, the user's psychical and physical characteristics (such as possible tolerance), and the environment of consumption. This is sometimes referred to as set and setting. Smoking the same cannabis either in a different frame of mind (set) or in a different location (setting) can alter the effects or perception of the effects by the individual. What the user does under the influence can also affect the effects of cannabis. For example, if

the user does nothing they will feel relaxed and sleepy, whereas if they engage in intense physical or psychical activity they will feel energised. Effects of cannabis consumption may be loosely classified as cognitive and physical. Anecdotal evidence suggests that drug varieties of *Cannabis sativa* subsp. *sativa* tend to produce more of the cognitive or perceptual effects, while *C. sativa* subsp. *indica* tends to produce more of the physical effects.

Figure 5. A typical utensil use for smoking marijuana.

ECS (endocannabinoid system)

The endocannabinoid system includes cannabinoid receptors, their endogenous ligands, anandamide and 2-arachidonoyl glycerol, the anandamide transporter protein, and two enzimes, fatty acid amide hydrolase (FAAH) and monoglyceride lipase (MGL).[3]

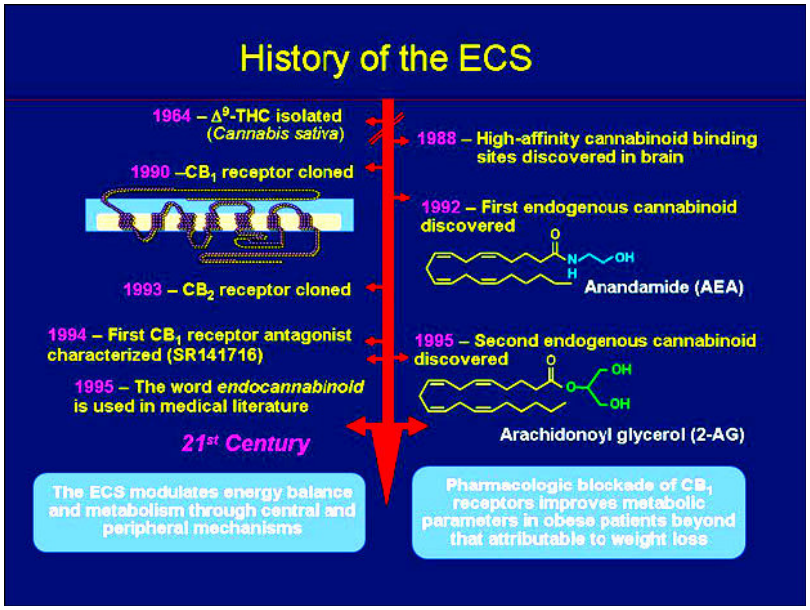


Figure 6

Cannabinoid receptors

Cannabinoid receptor types are denoted by the abbreviation CB and numbered in the order of their discovery by a subscript. At present, two cannabinoid receptor types have been unequivocally identified, named CB1 and CB2. These receptors belong to the super family of G-protein-coupled transmembrane receptors (GCPR) which are mainly expressed in the central

nervous system; especially in the hypothalamus, pituitary gland and in the mesolimbic dopamine circuits. It has detected not only in neurones but also in astrocytes and microglial cells.

It is also found in a variety of peripheral tissues such as adipose tissue, liver, muscle, the gastrointestinal tract, pancreas, urinary bladder, lung, adrenal gland, testis, ovary, prostate and in rat adipose tissue.

The CB2 receptors is limited essentially to the cells associated with the immune system, like spleen, thymus, tonsils, B cells and natural killer cells, monocytes, neutrophils and T cells.[4] Although absent from the CNS in normal conditions, CB2 receptors might be induced in brain microglial cells in response to different damaging conditions associated with local inflammatory events,[5] and recent studies using human neutrophils indicate that the CB2 receptors may suppress neutrophil migration during inflammation. It is also located in retina,[6] skin[7] and some malignant cells.[8]

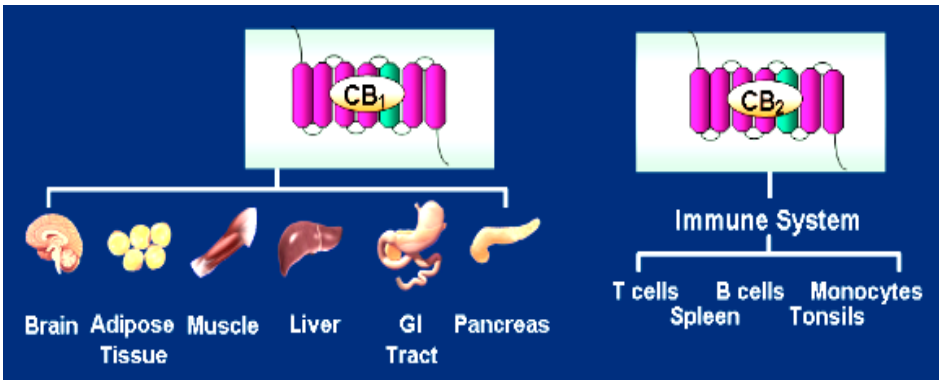


Figure 7

The human CB1 and CB2 receptorss exhibit 68% identity within the transmembrane regions, 44% identity throughout the whole protein. A new putative cannabinoid receptor gene has been recently identified in the invertebrate *Ciona intestinalis* that share 28% sequence identity with the human CB1 and 24% sequence identity with the human CB2.[9]

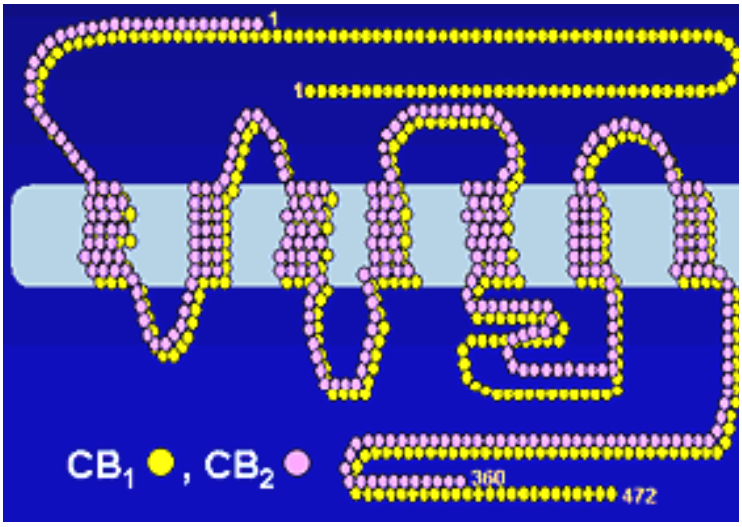


Figure 8

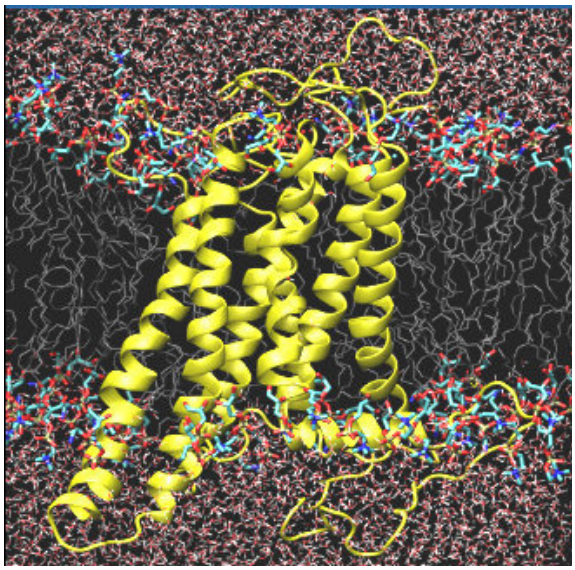


Figure 9

Endogenous ligands of cannabinoid receptors

The individuation of endogenous ligands for these receptors has been yielded after cannabinoid receptors discovery. The endogenous ligand of the CB1 receptors is anandamide whereas the endogenous ligand of the CB2 is 2-arachidonylglycerol (2-AG) (Figure 10). They are synthesized from membrane-derived phospholipids whose biologic effects are mediated through coupling with the specific, widely expressed ECS receptors located presynaptically.[10] The synthesis of anandamide is Ca^{2+}-dependent and is produced locally by the phospholipase D-mediated cleavage of the membrane precursor called N-arachidonoyl-phosphatidylethanolamine (NArPE). While the synthesis of 2-arachidonylglycerol is produced by the diacylglycerols (DAG) lipase cleavage of the membrane precursor. Because endocannabinoids are lipophilic compounds derived from membrane phospholipids, they do not need to be stored in synaptic vesicles like other neurotransmitters.[11] In the brain, they are produced by neurons at their sites of action and act on demand, generating a transient, rapid effect before being hydrolyzed and inactivated by fatty acid amide hydrolase (FAAH), which breaks the amide bond and releases arachidonic acid and ethanolamine.[10, 11] Because of their lipophilic nature and the mechanism of their synthesis and release, endocannabinoids are considered as local neuromodulators.[12]

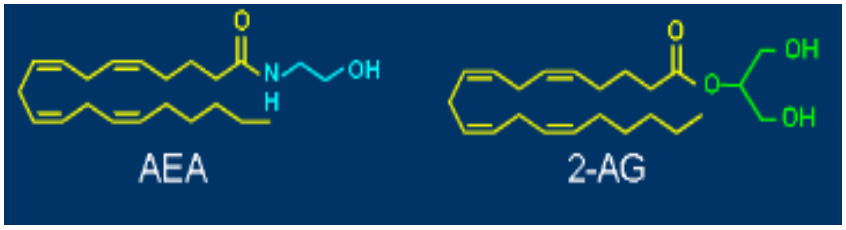


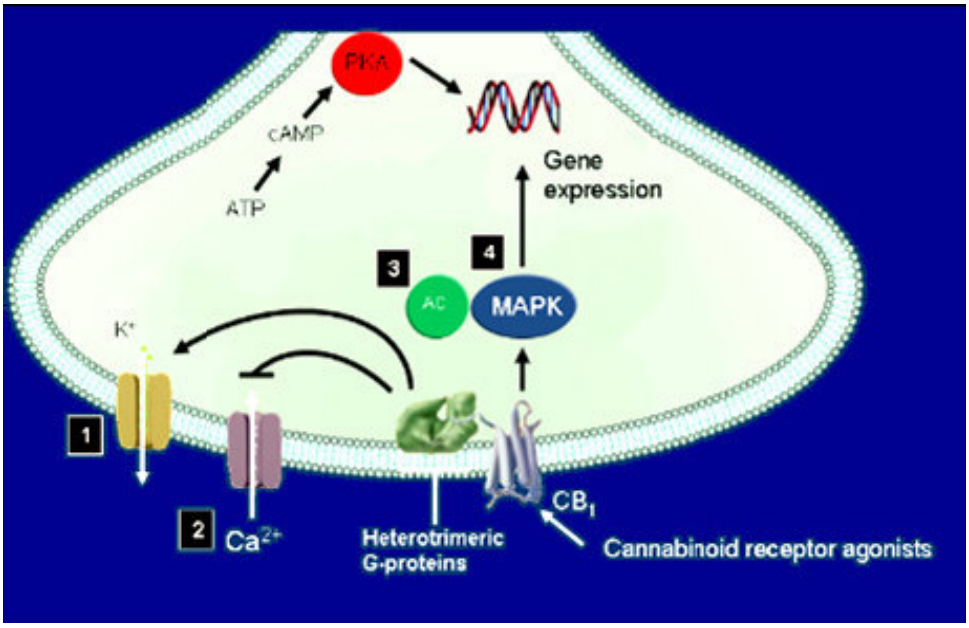
Figure 10

Signal transduction activated by cannabinoid receptors

The protein G, which is coupled to receptor, is constituted by the α and βγ subunits and the G protein (Gi/Go) associated to cannabinoid receptors is sensible to pertussis toxin. The affinity of CB1 and CB2 for Gi or Go proteins may be different as revealed by several studies on cannabinoid ligand binding or regulation of [35s] GTPγS binding. Whereas, activation of both of them display a high affinity for Gi, agonist stimulation of CB1 also result in a high-affinity saturable interaction with Go but CB2 receptors do not interact efficiently with Go. It has been reported that the affinity of CB1 receptors for Go is ten fold higher than that of the CB2.[13] It has been described that the juxtamembrane C-terminal region of CB1 receptorss (amino acids 401-417) and the second and third intracellular loops are critical for Gi/o protein coupling and that the distal C-terminal tail domain profoundly modulates both the magnitude and kinetics of signal transduction.[14] In the CB2 receptors it has been described the existence of two cysteins, C313 and C320, that are located in this C-terminal region, that may play important roles for receptor-G protein coupling and receptor desensitization. Also, the third transmembrane domain in the CB2, particularly the Asp-Arg-Tyr motif, may be crucial for interacting with G proteins.

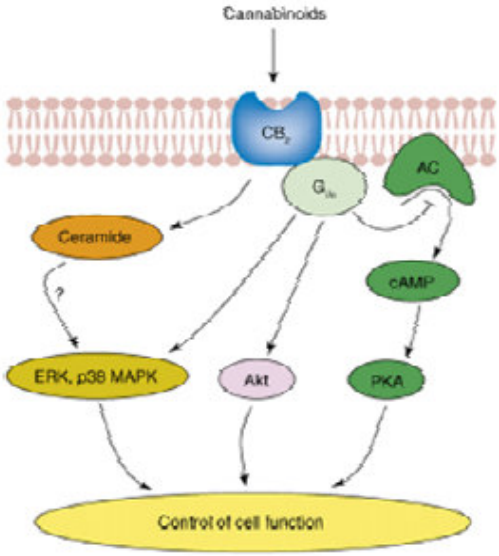
Some effects are independent by interaction with G proteins, these are negative regulation of adenylate cyclase and the cAMP/protein kinase A (PKA)-dependent pathway. An effect mediated by G protein is inhibition of calcium channels. In fact cannabinoid agonists reduce the amplitude of voltage-gated calcium currents in neuronal cells through Gi/o proteins. Cannabinoids also modulate K^+ channels and cannabinoids as THC cause a depression of inward sodium current determining a depression of action potentials.[15, 16] CB2 receptors seems to be independent of channel activation.

These GPCRs (G protein-coupled receptors) can stimulate the mitogen-activated protein kinase (MAPK) cascade and induce cellular proliferation. The mammalian MAPK family consists of three subfamilies with multiple members: the extracellular signal-regulated kinases (ERK), the Jun amino-terminal kinases/stress-activated kinases (JNK/SAPK), and the p38 MAPKs. While ERK is involved in regulation of cell division and growth, the other two subfamilies are activated by stress signals and inflammatory cytokines and have been related with cellular death and immune disorders. In addition, the activation of CB2 receptorss has been also linked to the stimulation of additional intracellular pathways including the phosphatidylinositol 3-kinase (PI3K)/Akt pathway, which has been associated with pro-survival effects, and the *de-novo* synthesis of the sphingolipid messenger ceramide, which has been linked with the pro-apoptotic effects of cannabinoids (Figure 11-12).



Signal transduction activated by CB_1 receptors.

Figure 11



Signal transduction activated by CB_2 receptors.

Figure 12

Role of cannabinoids

For over 4000 years, *Cannabis sativa L* extract have been widely used as recreational drug and as therapy for a variety of disorders. The discovery of psychoactive principle of *Cannabis sativa,* Δ^9-tetrahydrocannabinol initiated research into the physiological role of cannabinoids. In the last years our understanding has been modified significantly by new discovery.

Cannabinoids are best known for their effects on CNS functions. They produce euphoria, alterations in cognition and analgesia, have anticonvulsant properties and affect temperature regulation, sleep and appetite. However, cannabinoids also possess immunomodulatory activity and anti-inflammatory properties. Many diseases of the CNS involve inflammation, and cause an up regulation of cytochines and other inflammatory mediators in the CNS. Alzheimer's disease (AD), Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS) are among the best examples of neurodegenerative disorders of the CNS associated with intense inflammation, whereas multiple sclerosis (MS) and HIV-associated dementia are inflammatory disorders of the CNS that lead to diffuse neuronal damage.

Therefore cannabinoids may be potential therapeutic agents in neurological diseases. Recent studies revealed mainly role of cannabinoids in:

- Cognition: the most prominent among the various consequences of CB1 receptors activation by exogenous agonist are disruptive effects on working memory, i.e. on processes necessary to learn and react to new information that differs from session to session.
- Emotionally: blockade of CB1 receptorss by SR141716A (CB1 agonist) caused an increase in anxietyrelated behavior.[17, 18, 19] In contrast, lower doses of SR141716A had no effects.[20] Data obtained in mice were more inconsistent. Administration of SR141716A either decreased[21] or increased anxiety-related behaviour, depending on the genetic background of animals and the test situation.

- Multiple sclerosis (MS): is the most important chronic inflammatory demyelinanting disorder of the CNS. The cannabis and cannabinoid agonists may be effective in ameliorating symptomatology of MS, especially spasticity and pain. The benefits of cannabinoids agonist the symptomatology associated with chronic inflammatory demyelinanting pathologies, may be exert at multiple levels: by improving motor function, by limiting neuroinflammation, by promoting remyelination.
- Immune modulation: the cannabinoid CB2 receptors is expressed abundantly in various types of inflammatory cells in particular in B cells. The CB2 receptorss has been associated with most of immunomodulatory activity of cannabinoids, but also CB1 may be linked to cannabinoid-mediated alterations of immune cell reactivity. Cannabinoids exhibit immunosopressive properties.
- Appetite and energy regulation: the CBr is postulated to connect the physical and emotional responses to stress with appetite and energy regulation (general stress-recovery system). Stimulation of the CBr may possibly occur as a consequence of obesity, leading to increased levels of endocannabinoids, which disrupt the feedback mechanism involved in energy balance. The CBr affects energy balance, glucose homeostasis, and lipogenesis because of the cannabinoids receptors are located in the adipose tissue, the liver, the pancreas and the skeletal muscle.
- Obesity: activation of the CBr increases food intake and promotes weight gain. The blockade of the CB1 receptors reduces body weight in animals through central and peripheral action.

Pharmacological role of cannabinoi receptors

The physiological and therapeutic potential of the CB2 receptors largely remains unexplored; however, recent data indicate that CB2 cannabinoid receptors participate in the control of peripheral pain, inflammation, osteoporosis, growth of malignant gliomas, tumors of immune origin, and immunogical disorders such as multiple sclerosis. The activation of the cannabinoid signaling system, in discrete brain regions after pathological neuroinflammatory insults, represents an endogenous response of the brain to maintain nerve cell homeostasis and to reduce the injury associated with conditions of excitotoxicity, inflammation, trauma, infection and other types of neurotoxic stimuli.

Pain

The use of cannabis has been described in classical and recent literature for the treatment of pain, but the potential for psychotropic effects as a result of the activation of central CB1 receptorss places a limitation upon its use; there are, however, a number of modern approaches being undertaken to circumvent this problem. Selective CB2 agonists and peripherally restricted CB1 or CB1/CB2 dual agonists are being developed for the treatment of inflammatory and neuropathic pain, as they demonstrate efficacy in a range of pain models. CB2 receptorss were originally described as being restricted to cells of immune origin, but there is evidence for their expression in human primary sensory neurons, and increased levels of CB2 receptorss reported in human peripheral nerves have been seen after injury, particularly in painful neuromas.

CB2 receptors agonists produce antinociceptive effects in models of inflammatory and nociceptive pain, and in some cases these effects involve activation of the opioid system. In addition, CB receptor agonists enhance the effect of μ-opioid receptor agonists in a variety of models of analgesia,

and combinations of cannabinoids and opioids may produce synergistic effects. Antinociceptive effects of compounds blocking the metabolism of anandamide have been reported, particularly in models of inflammatory pain. There is also evidence that such compounds increase the analgesic effect of non steroidal anti-inflammatory drugs (NSAIDs), raising the possibility that a combination of suitable agents could, by reducing the NSAID dose needed, provide an efficacious treatment strategy, while minimizing the potential for NSAID-induced gastrointestinal and cardiovascular disturbances. Other potential "partners" for endocannabinoid modulatory agents include α_2-adrenoceptor modulators, peroxisome proliferatoractivated receptor α agonists and TRPV1 antagonists. An extension of the polypharmacological approach is to combine the desire pharmacological properties of the treatment within a single molecule. Hopefully, these approaches will yield novel analgesics that do not produce the psychotropic effects that limit the medicinal use of cannabis.[22]

It has been well established that the ability of cannabinoids to affect pain perception has supra-spinal, spinal and peripheral components.[23] With respect to the peripheral component, local administration of both synthetic cannabinoids and exogenous anandamide and 2-AG produce antinociceptive effects in the formalin model of inflammatory pain.[24]

Fride et al. (2004)[25] have identified analog s of the (+)-enantiomer of cannabidiol that are active towards CB receptors and reduce the pain response in the formalin model of inflammatory pain, without producing overt signs of central CB1 receptors activation. Other peripherally restricted CB1 or CB1/CB2 receptors agonists are currently being investigated as potential approaches to the treatment of pain. A good example of this is the study of Dziadulewicz et al. (2007)[26]. These authors reported that naphthalen-1-yl-(4-pentyloxynaphthalen-1-yl)methanone bound as an agonist to human CB1 and CB2 receptorss with IC_{50} values of 15 and 98 nM, respectively, and produced a good separation of effects upon

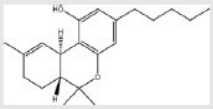
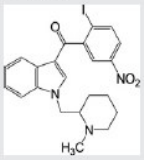
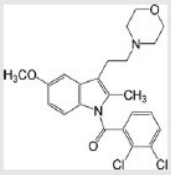
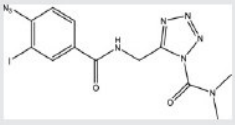
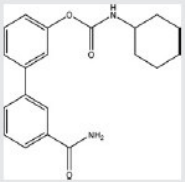
neuropathic pain and catalepsy, consistent with a limited penetration of the compound into the brain.

Inflammation

Activation of cannabinoid CB1 and CB2 receptors subtypes suppresses pain behaviour resulting from tissue injury and inflammation. The ability of peripheral cannabinoid mechanisms to modulate the development of inflammatory nociception is well established, less is known about peripheral cannabinoid antihyperalgesic mechanisms after the establishment of chronic inflammation. Local administration of agonist suppressed tactile allodynia and mechanical hyperalgesia with the expected pharmacological specificity. However, antihyperalgesia efficacy and pharmacological specificity for the CB2 selective agonist was less robust in tests of thermal compared to mechanical hypersensitivity. Local administration of cannabinoids suppress capsaicin-evoked calcitonin gene-related peptide release in rat spinal cord in vitro,[27] suggesting a possible neuronal mechanism of action. CB2 receptors protein has been identified in microglial cultures of neonatal rat spinal cord,[27] suggesting the existence of additional non-neuronal substrates capable of mediating the antihyperalgesic actions. Activation of CB2 receptors on skin keratinocytes stimulates production of β-endorphin to induce antinociception through activation of μ-opioid receptor.[28] Locally administered CB1 and CB2 selective agonists (respectively ACEA and AM 1241) induced qualitatively similar suppressions of allodyna and hyperalgesia. Local administration of either ACEA and AM1241 at site of inflammation may suppress antihyperalgesic efficacy by reducing primary afferent sensitization, effects consistent with the observation that cannabinoids suppress capsaicin-evoked calcitonin gene-relatedpeptide release.[27,29] Carrageenan also enhances C-fibre-mediated responses and windup in spinal dorsal horn

neurons effects, that enhance spinal neuronal excitability. These effects are also modulated by both CB1 [30] and CB2 specific mechanisms.[31]

Table 1. The pharmacology of some of the CB1/CB2 selective compounds.

Compound	Mechanism of action	Effect in pain
THC 	Primarily activation of CB receptors, although has off-target actions (e.g. Barann et al., 2002)	Inflammatory: + Neuropathic: +
AM1241 	CB_2-receptor selective ligand; acts as a "protean" agonist *in vitro* (Yao et al., 2006) and CB2 agonist *in vivo*	Inflammatory: + Neuropathic: +
GW405833 	CB_2-receptor selective ligand (efficacy dependent upon assay used, see Yao et al., 2008)	Inflammatory: + Neuropathic: +
LY2318912 	Blocks the accumulation and metabolism of AEA (Moore et al., 2005). Acts primarily as a potent FAAH inhibitor, but with many off-target actions (Alexander and Cravatt, 2006)	Inflammatory: +
URB597 	Selective FAAH inhibitor (Kathuria et al., 2003). Some off-target actions have been reported, but their importance is unclear	Inflammatory: + Visceral: + Neuropathic: +/−

Neuroprotection

Recent studies have now implicated CB2 receptorss in neuroprotective activity of cannabinoids by blocking of microglia activation (Figure 13), mainly through a series of glia-dependent anti-inflammatory actions.[32] Microglia have an active role in the defensive attack against viruses and bacteria, but intense microglial activation, such as that produced to clear up

apoptotic cells or neuron debris during neurodegenerative disorders, can be detrimental to the survival of neighboring cells. Substantial in vivo and in vitro results show that cannabinoid protect neurons from death.[32, 33, 34] This neuroprotection might be relevant for the treatment of both acute brain injury (e.g. cerebral ischemia and trauma) and chronic neurodegenerative disorders (e.g. Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis and multiple sclerosis).

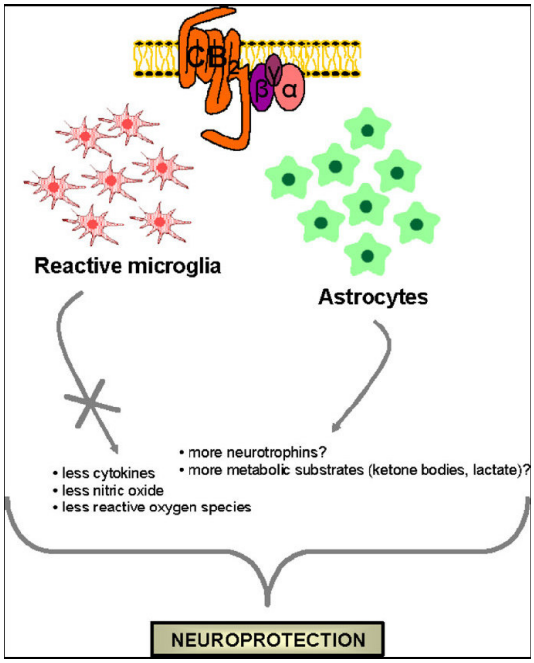


Figure 13

Amyotrophic lateral sclerosis (ASL): is a neurodegenerative disease characterized by rapid progressive degeneration of motor neurons in the brain and spinal cord, paralysis and death within 2-5 years from the diagnosis. ASL is the third most common neurodegenerative cause of adult death, after Alzheimer' s disease and Parkinson's disease. Most cases of ASL are sporadic and are probably acquired, while approximately 10% are familial. Despite a variety of putative underlying oxidative stress,

neuroinflammation, autoimmunity, a defect in neuronal glutamate transport, glutamate toxicity and mutations of superoxide dismutase gene. Recent evidence indicates that ASL is a disease characterized by chronic inflammation.[35, 36] Microglia are the resident macrophages of CNS and in response to CNS injury microglia quickly convert to on "active" state during which they change to on amoeboid shape, up-regulate the cell-surface expression of a variety of surface antigens and secrete several pro-inflammatory molecules[37] including interleukin-1β, tumour necrosis factor-α, nitric oxide,[37] oxygen radical, glutamate, proteases and contribute to the pathogenesis of neurologic disorders. The microglial activation in the CNS suggest a primary neuroinflammatory state with deleterious effects on surrounding neurons.[38] Recent study reported elevated levels of CB2 receptorss in microglia isolated from post-mortem human spinal cord of ASL patients[39] moreover recently in vitro studies demonstrate that CB2 receptorss are up-regulated in microglia in response to inflammatory stimuli[40] and that CB2 agonist suppress microglial activation.[41] In 2005 Jared Ehrart et al showed that the CB2 agonist JWH 015 inhibits INF-γ-induced microglial CD40 expression. They proved that when CB2 is stimulated by the presence of JWH 015 the pro-inflammatory molecules were significantly reduced. Recent studies demonstrate that treatment at onset with the non selective CB1/CB2 agonist WIN 55-212 produces a significant rightward shift in the survival curve on G93A mice reflected by an increase of 8.8 days in the survival interval.

Administration of the selective CB2 agonist AM1241 results in a highly significant extension of survival with mice living 56% longer after symptom onset than controls. These results suggest that CB2 agonist may be effective as pharmacological agents with several distinct advantages for the management of this devastating disease.[42]

Multiple sclerosis (MS): the anti-inflammatory properties of cannabinoids against MS pathophysiology seem to be associated with a reduced number

of activated microglia in the spinal cord, decreased expression of major histocompatibility complex class II, and decreased number of $CD4^+$ T-cells, as described in models of MS. Decreased synthesis of proinflammatory cytokines are other important effects of these compounds in mice with experimental MS. Activation of CB2 receptorss inhibits the production of several inflammatory cytokines, cell proliferation and chemiotaxis, down-regulates macrophage function, inhibits microglial activation and limits nitric oxide release (Figure 14). All these factors are critical determinants for the maintenance of inflammation in MS. It should be mentioned, however, that activated microglia and macrophages express not only CB2 but also CB1 receptorss, suggesting that both receptor subtypes might be important for the anti inflammatory effects of cannabinoids in MS. A report also allowed to extend to non-CB1 and non-CB2 receptorss the inhibitory action of cannabinoids on astrocyte activation, suggesting a role for these receptors in the anti-inflammatory effects of exogenous and endogenous cannabinoids in MS and experimental MS. A portion of the neurodegenerative damage seen in MS results from the release of toxic cytokines and free radicals by activated microglia or other immune cells invading the CNS. Thus endocannabinoids, by inhibiting inflammation, also exert an indirect neuroprotective action in MS. Cannabinoids, however, exert direct neuroprotective effects by limiting glutamate release and excitotoxic damage in several neurodegenerative disorders by acting through CB1 receptorss. Excitotoxicity plays a crucial role also in MS-associated neuronal damage, suggesting that pharmacological stimulation of CB1 may contrast neurodegenerative damage also in this disorder.[43]

Cannabinoids suppress behavioural responses to noxious stimulation and suppress nociceptive transmission through activation of CB2 receptors subtype. Indeed, activation of CB2 receptorss on non-neuronal cells has been postulated to suppress the release of inflammatory mediators that sensitize nociceptors. Thus, non-neuronal substrates as well as neuronal substrates may be responsible for the ability of CB2-selective agonists to

suppress persistent pain states.[44] These mechanisms may also contribute to the more pronounced effects of selective CB2 agonists in inflamed compared to non-inflamed tissue.

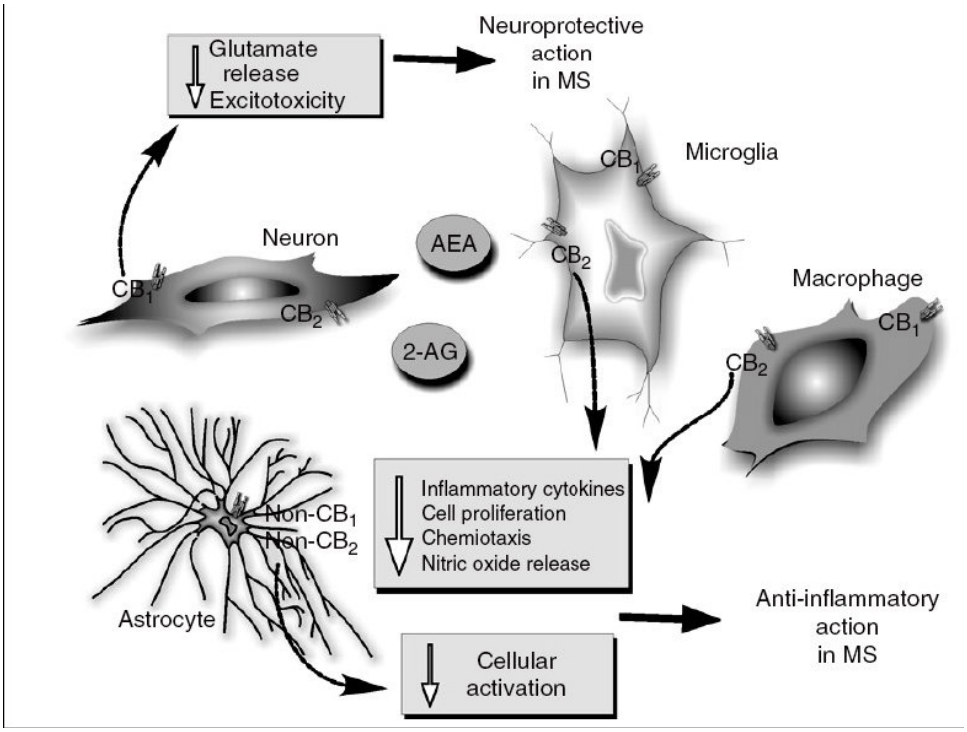


Figure 14

Glaucoma

Experimental findings indicate that the cannabinoids (CBs) could be use for treatment of glaucoma, because endocannabinoid system contributes to the control of intraocular pressure (IOP), by modulating both production and drainage of aqueous humor. There is also a growing body of evidence of the involvement of this system in mechanisms leading to the death of retinal ganglion cells, which is the end result of glaucoma.

Glaucoma is an optic neuropathy characterized by apoptotic death of the retinal ganglion cells (RGCs) and loss of the axons that make up the optic

nerve.[45] The changes provoked by glaucoma are progressive, and if left untreated can produce severe visual-field deficits.

The pathogenesis of glaucoma is complex and multifactorial, but elevated intraocular pressure (IOP) is one of the risk factors most closely associated with both onset and progression of the disease. Increases in the IOP are believed to be responsible for blood-flow alterations that ultimately lead to hypoxia and ischemia of the retina and the optic nerve. Treatment — medical or surgical — is aimed at reducing IOP to prevent structural and functional damage, but this approach is not always sufficient to block progression of the disease and to prevent blindness.[46] Progressive optic nerve damage has in fact been documented in many glaucoma patients, whose IOP has been pharmacologically or surgically normalized,[47] and in others whose untreated pressure is well within the normal range (normal tension glaucoma).[48] Therefore, it seems clear that the damage caused by glaucoma is related in part to other factors (vascular, genetic, etc.) that have yet to be identified. Experimental data suggest that the excitotoxic cascade triggered by glutamate plays a fundamental role in RGC damage associated with glaucoma.[49] Elevated intravitreal levels of this neurotransmitter have been documented in experimental models of glaucoma[50] and in glaucoma patients.[51]

A great deal of information concerned with the interplay between the glutamanergic and endocannabinoid system have been recently accumulated in the normal and pathological eye and these open new lanes of investigation.

Earlier studies demonstrated that CB1 receptors are located in the eye[52] and that functional CB2 receptors are also expressed in the retina and trabecular meshwork.[53]

The discovery by Straiker et al. (1999)[54] of CB receptors and their agonists at the ocular level sparked numerous attempts to determine whether and how this system might be involved in physiologic andor pathologic processes of the eye. There is a large body of experimental data showing

that certain endocannabinoids and synthetic CBs can reduce IOP, and these findings have led to the suggestion that CBs might be used in the treatment of glaucoma, together with or instead of traditional antiglaucoma drugs (α_2-agonists, carbonic anhydrase inhibitors, prostaglandin analogs, β-blockers)[55]. Additional support for the use of this approach has emerged from more recent studies, which indicate that the (endo)cannabinoids are also capable of producing specific neuroprotective effects at the level of the retina.

The first studies on the IOP-reducing effects of CBs date back to the 1970s. Hepler and Frank (1971)[56] found that eating or smoking marijuana reduced IOP by 5–45% (mean, approximately 25%). However, since the effect lasted for only 3–4 h, patients would have to use cannabis 8–9 times a day to keep their IOP under control. The beneficial effects of marijuana on ocular hypertension were also accompanied by several toxic effects, including orthostatic hypotension, increased heart rate, emphysema-like lung changes, diverse alterations of the mental state (euphoria, reduced attention, short-term memory deficits, and altered motor coordination), and at the ocular level they included conjunctival hyperemia associated with a 50% reduction in tear secretion.[57]

Porcella et al. (2001)[58] reported that WIN-55-212-2, a synthetic CB that binds CB1 and CB2 receptors, decreases IOP in patients with a type of glaucoma that was refractory to conventional treatment. Endocannabinoids like AEA[59] or noladin ether[60] have also been found to reduce IOP without producing systemic toxic effects.

In a recent pilot study, sublingual administration of THC reduced IOP in patients with glaucoma, without producing any significant systemic side effects.[61] It seems clear that, while the use of marijuana for the treatment of glaucoma is not supported by scientific evidence, other molecules — natural and synthetic — that interact with the ocular endocannabinoid system are offering new perspectives for the control of IOP. In addition, Lograno and Romano (2004)[62] showed that activation of the CB1 receptors

by AEA or CP55,940 caused contraction of the ciliary muscle, an event known to promote outflow of the aqueous humor through the trabecular meshwork.

Experimental findings support the view that drugs capable of interacting with the endocannabinoid system exert specific neuroprotective effects.[63]

The neuroprotective effects of endocannabinoids (eCBs) might also be related to their ability to modulate inflammatory processes. In glaucoma, post-ischemic inflammation is thought to be an important factor in the induction of cell death. Experimentally induced glaucoma has been shown to be associated with massive activation of astrocytes, Muller cells, and microglia that leads to the release of factors that are toxic to the ganglion cells, including nitric oxide, glutamate, and tumor necrosis factor.[64]

Experimental studies have also shown that stimulation of CB1 and CB2 receptors modulates activation and migration of microglial cells, and thus inhibits the production of inflammatory cytokines and nitric oxide in models of CNS disease.[65]

The identification of plant derived, endogenous, or synthetic CBs capable of interacting with the intraocular endocannabinoid system could open new perspectives for the treatment of glaucoma. In addition to their ability to control IOP elevations caused by the disease, such agents could also produce specific neuroprotective effects at the level of retinal ganglio cells, whose loss represents the final event along with disease progression. In ophthalmology, the risk of serious systemic side effects could be substantially reduced by the use of eye drops containing (endo)cannabinoids, although topical application is complicated by the markedly low water solubility of these molecules. The recent introduction of microemulsions and cyclodextrins as vehicles has considerably increased the ability of (endo)cannabinoids to diffuse across the cornea, thus enhancing their effects on the IOP.[65a]