**REVIEW**

# Cannabidiol – Recent Advances

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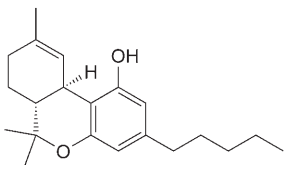
The aim of this review is to present some of the recent publications on cannabidiol (CBD; **2**), a major non-psychoactive constituent of Cannabis, and to give a general overview. Special emphasis is laid on biochemical and pharmacological advances, and on novel mechanisms recently put forward, to shed light on some of the pharmacological effects that can possibly be rationalized through these mechanisms. The plethora of positive pharmacological effects observed with CBD make this compound a highly attractive therapeutic entity.

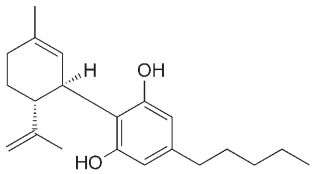
## Contents

**1. Introduction.** – Over the last 40 years, cannabinoid chemistry and pharmacology have been the object of thousands of publications. For obvious reasons, most attention has been paid to $\Delta^9$-tetrahydrocannabinol (THC; **1**), the psychoactive constituent of Cannabis. As cannabidiol (CBD; **2**)[1]), a non-psychotropic plant constituent, is generally found in relatively high concentrations in Cannabis, several groups have also investigated the pharmacological activities of this component, although not to the extent that THC was investigated. Over the last decade, however, interest in CBD has increased considerably.

Several groups have summarized the knowledge gathered on CBD up to the last few years [1–5]. We have published two concise reviews, the first dealing with the chemistry of CBD [6], and the second related to its pharmacology and biological effects [7]. The aim of the present review is to collect some of the recent publications, to give a general overview of the field, with emphasis on biochemical/pharmacological advances as well as on novel mechanisms recently revealed, and to try to shed light on some of the pharmacological effects that can possibly be rationalized through these mechanisms.




THC (**1**)




CBD (**2**)

**2. Mechanisms of Cannabidiol Action.** – CBD (**2**) does not cause marijuana-like effects. However it has been shown to produce a plethora of pharmacological effects, many of them associated with both central and peripheral actions (see below). Until recently, very little was known about the biochemical/physiological basis of these activities. Over the last two years, several groups have reported some unexpected observations.

2.1. *Cannabidiol: an Antagonist of $CB_1$- and $CB_2$-Receptor Agonists.* CBD (**2**) has a very low affinity for both known cannabinoid receptors, $CB_1$ and $CB_2$. However, *Pertwee*'s group in Aberdeen showed that CBD antagonizes the $CB_1$ agonists and noradrenaline in mouse *vas deferens*, a tissue in which prejunctional $CB_1$ receptors mediate inhibition of electrically evoked contractions by suppressing noradrenaline and ATP release [1]. CBD attenuated the ability of the $CB_1$ agonists WIN55212 and CP55940 to affect contractions at doses considerably lower than those of CBD needed to activate cannabinoid receptors. Their conclusion was that CBD antagonizes the above two cannabinoid agonists by acting at prejunctional sites that are unlikely to be $CB_1$ or $CB_2$ cannabinoid receptors. In a more recent publication, the Aberdeen group reported that these actions are actually cannabinoid-receptor-mediated [8]. They found that CBD is a high-potency antagonist of cannabinoid-receptor agonists in

[1]) Systematic name: 2-[(1*R*,6*R*)-3-methyl-6-(1-methylethenyl)cyclohex-2-en-1-yl]-5-pentylbenzene-1,3-diol.

mouse brain and in membranes from cells transfected with human $CB_2$. CBD was also found to display inverse agonism at the human $CB_2$ receptor. These unexpected observations may rationalize many of the effects recorded with CBD, such as its anti-inflammatory properties.

2.2. *Cannabidiol: Enhancer of Adenosine Signaling*. On examining the effects of CBD (**2**) on microglial proliferation, the *Hillard* group in Wisconsin found that CBD potently inhibits [$^3$H]thymidine incorporation into a murine microglial cell line, with no effect on the cell cycle [9]. Treatment with CBD decreased [$^3$H]thymidine uptake into microglia, with an $IC_{50}$ value matching inhibition of [$^3$H]thymidine incorporation into DNA. CBD decreased uptake of [$^3$H]adenosine to a similar extent as [$^3$H]thymidine in both murine microglia and RAW264.7 macrophages. Binding studies confirmed that CBD binds to the equilibrative nucleoside-transporter-1, with a $K_i$ value below 250 nM. It seems reasonable to assume that CBD is immunosuppressive, because it enhances endogenous adenosine signaling. *In vivo* treatment with a low dose of CBD is known to decrease TNF-$\alpha$ production in lipopolysaccharide (LPS)-treated mice [10]; this effect is reversed with an A2A adenosine-receptor antagonist, and abolished in A2A receptor knockout mice. Thus, it was demonstrated that CBD has the ability to enhance adenosine signaling through inhibition of uptake and provide a further non-cannabinoid-receptor mechanism by which CBD can decrease inflammation.

2.3. *Cannabidiol: Action on the 5-HT1a Receptor*. CBD (**2**) displaces the agonist '[$^3$H]-8-hydroxy-2-di-*n*-propylamino-tetralin' ([$^3$H]-8-OH-DPAT) from the cloned human 5-HT1a receptor in a concentration-dependent manner [11]. In contrast, THC (**1**) does not displace this agonist from the receptor in equivalent micromolar concentrations. CBD is a modest-affinity agonist at the human 5-HT1a receptor; however, CBD increases [$^{35}$S]GTP$\gamma$S binding in this G-protein-coupled receptor (GPCR) system, as does the agonist serotonin. In addition, in this GPCR system, which is negatively coupled to cAMP production, both CBD and 5-hydroxytryptamine decrease the cAMP concentration at similar apparent levels of receptor occupancy [11].

As described below, CBD significantly reduces the infarct volume induced by middle-cerebral-artery (MCA) occlusion in a bell-shaped curve. This neuroprotective effect of CBD was inhibited by WAY100135, a serotonin (5-HT1A)-receptor antagonist, but not by capsazepine, a vanilloid-receptor antagonist. The cerebral blood flow, increased by CBD, was partially reversed by WAY100135. These results suggest that the neuroprotective effect of CBD may be related to the increase in cerebral blood flow through the serotonergic 5-HT1A receptor [12].

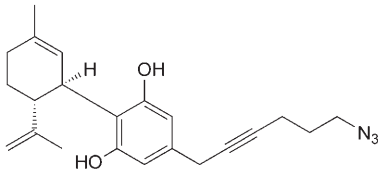
2.4. *Cannabidiol: a Potent Anti-Oxidant*. Phenols, including resorcinols, are well-known anti-oxidants. The plant cannabinoids, being monophenols, monophenolic ethers (like THC (**1**)), or resorcinols (as CBD (**2**)) are likewise potent anti-oxidants. *Eshar et al.* [13] found that HU-211, a (+)-THC-type cannabinoid, is a neuroprotective agent that combines NMDA-receptor antagonistic activity and free-radical-scavenging abilities in a single molecule. Following the same type of reasoning, *Hampson et al.* [14] investigated the anti-oxidative properties of CBD, and recorded that it prevents hydroperoxide ($H_2O_2$)-induced oxidative damage equally well, or better, than ascorbate (vitamin C) or tocopherol (vitamin E). Their data suggest that CBD may be a potentially useful therapeutic agent for the treatment of oxidative neurological disorders such as cerebral ischemia.

In a more recent study, *Hamelink et al.* [15] found that CBD, when administered concurrently with binge ethanol exposure in rats, protected against hippocampal-entorhinal-cortical neurodegeneration. They showed that this protection was not due to NMDA-receptor antagonism, as other NMDA antagonists that are not anti-oxidants did not prevent cell death, and attributed the CBD action to its anti-oxidative effects.

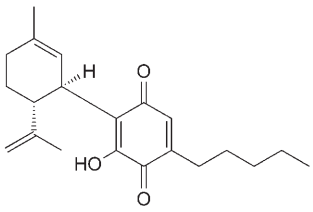
**3. Synthetic Approaches to Cannabidiol and Cannabidiol-Like Molecules.** – Recently, *Kobayashi et al.* [16] reported a new pathway for the synthesis of CBD (**2**) and its analogues. The key reaction was nickel (Ni)-catalyzed allylation of cyclohex-2-ene-1,4-diol monoacetate with a new reagent system, consisting of [Zn(alkenyl)Cl], *N*,*N*,*N*′,*N*′-tetramethylethylene-1,2-diamine (TMEDA), and catalytic amounts of $[NiCl_2(tpp)_2]$, which gave an $S_N2$-type product in good yield, and with 94% regioselectivity. While of considerable interest as a synthetic exercise, the new pathway is less facile than the previous one-step synthesis reported by *Baek et al.* [17].

Syntheses of both THC (**1**) and CBD (**2**) are by routes that lead either to racemates or require chiral precursors to obtain the natural products in their enantiomeric forms. *Trost* and *Dogra* [18] have shown that THC can be synthesized in enantiomerically pure form through molybdenum (Mo)-catalyzed asymmetric alkylation of a sterically congested doubly *ortho*-substituted entity. An important intermediate in their route was a hydroxylated dihydrocannabidiol, which presumably can be converted into CBD proper with synthetic ease. From a practical viewpoint, however, the new pathway is also less facile than the previous one-step synthesis of *Baek et al.* [17].

Agonists of the $CB_1$ cannabinoid receptor inhibit electrically evoked contractions of the *vas deferens* by mediating inhibition of the evoked release of the contractile neurotransmitters. CBD antagonizes this effect. *Thomas et al.* [19] reported the synthesis of the new CBD derivative '6″-azidohex-2″-yne-cannabidiol′ (O-2654; **3**)[2]), and compared it to CBD in antagonizing the effect of a $CB_1$ agonist on electrically evoked contractions of the *vas deferens*. Compound **3** was found to be as potent as CBD (**2**). However, it produced this antagonism with a potency that matched its affinity to the $CB_1$ receptor, suggesting that, unlike CBD, it acts as a competitive antagonist. Moreover, since it did not enhance the amplitude of electrically evoked contractions, it is presumably also a neutral $CB_1$ antagonist.




**3**




HU-331 (**4**)

[2]) Systematic name: 5-(6-azidohex-2-yn-1-yl)-2-[(1*R*,6*R*)-3-methyl-6-(1-methylethenyl)cyclohex-2-en-1-yl]benzene-1,3-diol.

Air oxidization of CBD in the presence of base leads to the cannabinoid 1,4-quinone HU-331 (**4**)[3]) [20]. This compound displayed antiproliferative activity in several human cancer cell lines, both *in vitro* and *in vivo*. Later, *Kogan et al.* [21] investigated its mode of action and presented evidence of a unique mechanism. HU-331 does not cause cancer-cell-cycle arrest, cell apoptosis, or caspase activation. Death of human cancer cells caused by HU-331 is not mediated by reactive oxygen species (ROS) or reactive oxygen intermediates (ROI), as exposure to **4** fails to elicit the generation of ROS. However, HU-331 specifically inhibits DNA topoisomerase II, even at nanomolar concentrations, but has only a slight, non-significant effect on the action of DNA topoisomerase I. The cannabinoid quinone HU-331 is a highly specific inhibitor of topoisomerase II, compared with most known anticancer quinones. Many of the quinonoid anticancer drugs cause heart damage. HU-331, in contrast, has no effect on the heart (unpublished results) and may, thus, represent a new potent anticancer drug.

We have described the syntheses of the major CBD metabolites (−)-7-hydroxy-cannabidiol (**5a**) and the corresponding carboxylic acid **6a**, their dimethylheptyl (DMH) homologues **5b** and **6b**, respectively, as well as the corresponding enantiomeric compounds in the (+)-CBD series [22][23]. The starting materials were the respective CBD enantiomers and their DMH homologues. The binding of these compounds to the $CB_1$ and $CB_2$ cannabinoid receptors were compared [24]. Surprisingly, contrary to the compounds in the (−)-CBD series, which do not bind to the receptors, most of the derivatives in the (+)-CBD series do bind to the $CB_1$ receptor in the low-nanomolar range; some of these compounds also bind weakly to the $CB_2$ receptor. Natural (−)-CBD and synthetic (+)-CBD, but not the other derivatives, also stimulate the type-1 vanilloid receptor. The (+)-CBD analogues that bind to the cannabinoid receptors exert peripheral pharmacological action only [25].

CBD (**2**) and its DMH analogue **7** were hydrogenated to afford the respective partly or fully hydrogenated epimeric compounds **8** and **9**, respectively. The new derivatives were evaluated for their ability to modulate the production of ROI, nitric oxide (NO), and tumor necrosis factor (TNF-$\alpha$) by murine macrophages, as well as for their binding to the $CB_1$ cannabinoid receptor. Surprisingly, we found that some of these derivatives exhibit good binding to $CB_1$. In addition, hydrogenated **2** and **7** exhibit bioactivities different from their parent compounds [26].

*Mannila et al.* [27] have used a precipitation/complexation method to prepare a complex of CBD (**2**) with $\beta$-cyclodextrin ($\beta$-CD). The effect of $\beta$-CD complexation on the sublingual absorption of CBD was studied in rabbits. The dissolution rate of the solid CBD–$\beta$-CD complex *in vitro* was significantly higher than that of free CBD ($p < 0.05$). These results demonstrate that the sublingual administration of solid CBD–$\beta$-CD enhances the absorption of CBD in rabbits, when compared to oral administration of CBD in EtOH solution. Thus, the solid CBD–$\beta$-CD complex may provide an alternative formulation for sublingual administration of CBD.

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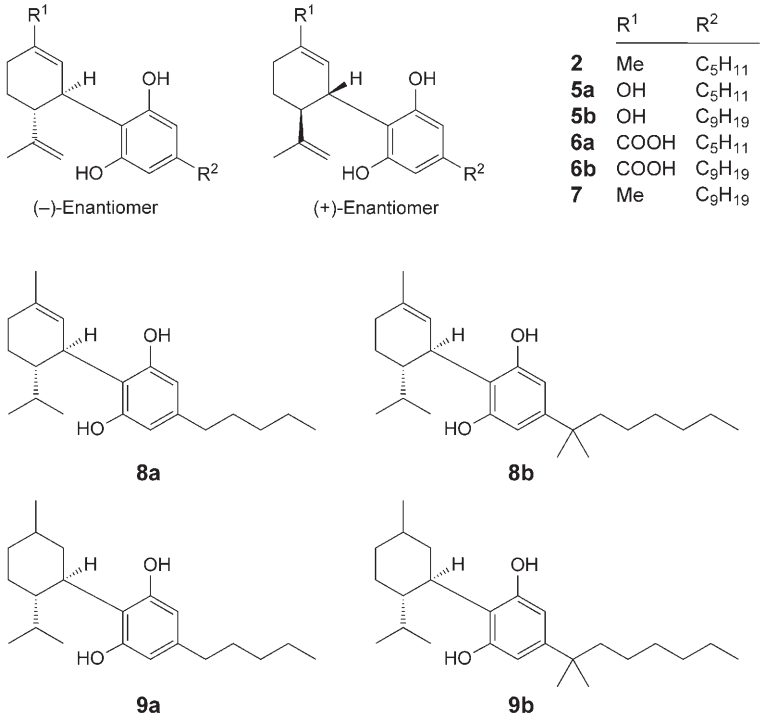
[3]) Systematic name: 3-hydroxy-2-[(1*R*,6*R*)-3-methyl-6-(1-methylethenyl)cyclohex-2-en-1-yl]-5-pentyl-cyclohexa-2,5-diene-1,4-dione.




| | $R^1$ | $R^2$ |
|---|---|---|
| **2** | Me | $C_5H_{11}$ |
| **5a** | OH | $C_5H_{11}$ |
| **5b** | OH | $C_9H_{19}$ |
| **6a** | COOH | $C_5H_{11}$ |
| **6b** | COOH | $C_9H_{19}$ |
| **7** | Me | $C_9H_{19}$ |

**4. Cannabidiol: Selected Biological Effects.** – 4.1. *Cannabidiol: an Allosteric Modulator of Opioid Receptors.* In view of the known non-competitive interaction between cannabinoid and opioid receptors, *Kathmann et al.* [28] examined by means of kinetics studies whether CBD (**2**) is an allosteric modulator at μ-opioid receptors, which are particularly sensitive for the measurement of allosteric interactions at G-protein-coupled receptors. In addition, they also studied whether such a mechanism also extends to the δ-opioid receptor. For comparison, THC (**1**) and rimonabant (a $CB_1$ cannabinoid-receptor antagonist) were also studied. In μ-opioid-receptor-binding studies on rat cerebral cortex membrane homogenates, the agonist [$^3$H]DAMGO bound to a homogeneous class of binding sites, with a $K_D$ value of $0.68 \pm 0.02$ nM. The dissociation of [$^3$H]DAMGO induced by naloxone (10 μM; half-life $t_{1/2} = 7 \pm 1$ min) was accelerated by both CBD and THC (100 μM each) by a factor of 12 and 2, respectively. The [$^3$H]DAMGO dissociation was not affected by rimonabant. In δ-opioid-receptor-binding studies on rat cerebral cortex membrane homogenates, the antagonist [$^3$H]naltrindole bound to a homogeneous class of binding sites, with a $K_D$ value of $0.24 \pm 0.02$ nM. The dissociation of [$^3$H]naltrindole, induced by naltrindole, was accelerated by CBD and THC (at 100 μM, each) by a factor of 2 each. [$^3$H]Naltrindole dissociation was not affected by rimonabant. This shows that CBD is an allosteric modulator at both μ- and δ-opioid receptors. This property is shared by THC, but not by rimonabant. The high levels of cannabinoids needed may, however, indicate that the actions are not physiologically relevant.

4.2. *Cannabidiol, Cytokines, and Related Endogenous Constituents.* Lymph-node cells (LNC) from mice treated with CBD (**2**) showed a diminished IFN-$\gamma$ release. In separate *in vitro* experiments, it was found that CBD suppressed the collagen-type-II-specific proliferation of LNCs from arthritic mice in a dose-dependent manner [10].

Synovial cells from mice that had been treated with an optimal dose of CBD (5 mg/kg i.p., daily, for 10 d) released significantly less TNF-$\alpha$, when cultured *in vitro*, than synovial cells from control animals. This finding suggests that the therapeutic action of CBD in arthritis includes the suppression of TNF-$\alpha$, a pro-inflammatory cytokine known to be a major mediator of this disease. This was corroborated by the finding that CBD, when injected intraperitoneal (i.p.) or subcutaneous (s.c.) at a concentration of 10 mg/kg, blocked lipopolysaccharide (LPS)-induced serum TNF-$\alpha$ in mice. However, there was no suppression of TNF-$\alpha$ release by arthritic synovial cells when CBD was added *in vitro*, nor could the authors demonstrate that CBD suppressed TNF-$\alpha$ release by mouse-bone-marrow-derived macrophages or RAW cells. This discrepancy between *in vivo* and *in vitro* results suggests that the TNF-$\alpha$ suppression, which is observed *in vivo* after administration of CBD, might be mediated by an active metabolite of CBD. Another possibility is that the decreased TNF-$\alpha$ expression *in vivo* is an indirect consequence of a suppressed T-helper-1 response. Whatever the mechanism of action, CBD exerts a potent immunosuppressive effect *in vivo*. Thus, the anti-arthritic potency of CBD seems to be the result of a combination of immunosuppression, especially of a T-helper-1 response, and an anti-inflammatory action by way of reducing TNF-$\alpha$ in the synovium, a combination that has proven successful in the past, when anti-IL-12 and anti-TNF-$\alpha$ entities were combined to treat collagen-induced arthritis. Apart from these major effects, the authors also demonstrated other *in vitro* anti-inflammatory actions of CBD that may contribute to its anti-arthritic potency, such as inhibition of the release of ROS by zymosan-stimulated neutrophils and blockade of NO production by peritoneal macrophages.

*Sacerdote et al.* [29] examined the *in vivo* and *in vitro* effect of CBD on the production of interleukins IL-12 and IL-10 by murine macrophages. CBD, when added *in vitro* to peritoneal macrophages, significantly increased IL-12 and decreased IL-10 production, respectively. Surprisingly, $CB_1$- and $CB_2$-receptor antagonists prevented this modulation. Macrophages from animals treated with CBD at a dose of 30 mg/kg, either orally or i.p., also produced higher levels of IL-12 and lower levels of IL-10, in comparison to controls, but the cannabinoid-receptor antagonists did not prevent these effects. These different effects by cannabinoid antagonists are difficult to rationalize.

Cannabinoids exhibit immunosuppressive actions that include inhibition of IL-2 production in response to a variety of T-cell-activation stimuli. Traditionally, the effects of these compounds have been attributed to the cannabinoid receptors $CB_1$ and $CB_2$, both of which are expressed in mouse splenocytes. Therefore, the *Kaminski* group investigated whether $CB_1$ and $CB_2$ antagonists affect the role of cannabinoid receptors in the cannabinoid-induced inhibition of IL-2 production stimulated by 'phorbol ester/calcium ionophore' (PMA/Io) in mouse splenocytes. PMA/Io-stimulated IL-2 production was, indeed, inhibited by CBD, which, however, was not attenuated by the presence of the cannabinoid antagonists [30].

The above publications support earlier, rather scarce reports on *in vitro* effects of CBD on immune cells, including the modulation of TNF-$\alpha$, IL-1, and IFN-$\gamma$ by human

peripheral-blood mononuclear cells [31][32] and the suppression of chemokine production by a human B-cell line [33].

4.3. *Cannabidiol and Sleep*. It is well-established that THC (**1**) increases sleep [34]. However contradictory reports have been published regarding the action of CBD (**2**). An early publication [35] reported biphasic effects in rats: at 20 mg/kg, a decrease in slow-wave sleep (SWS) was observed; but at 40 mg/kg, the SWS time was increased, while wakefulness was decreased. Tolerance developed rapidly. Human volunteers, receiving large doses of CBD (160 mg), spent significantly more time asleep than those receiving placebo [36]. In a recent clinical double-blind trial, 15 mg CBD administered with an oromucosal spray '*appears to have alerting properties as it increased awake activity during sleep and counteracted the residual sedative activity of 15 mg THC*' [37]. *Murillo-Rodriguez et al.* [38] reported that intracerebroventricular (icv) administration of CBD (10 μg/5ml) at the beginning of a lights-on period increased wakefulness and decreased rapid-eye-movement (REM) sleep. Enhancement of c-Fos expression was apparent in waking-related areas in the brain (hypothalamus and dorsal raphe nucleus). Extracellular levels of dopamine, serotonin, and noradrenaline were increased within the *nucleus accumbens*, whereas the extracellular levels of 3,4-dihydroxy-L-phenylalanine (L-DOPA) and '5-hydroxy-indole-acetic acid' (5-HIAA) were diminished. None of these effects were found during the lights-off period.

Surprisingly, the endocannabinoid anandamide, which is known to induce sleep, did not block the effect of CBD. The mechanism of CBD induction of wakefulness is unknown. It could include changes in dopamine (DA) levels, as the nigrostriatal dopaminergic system has been pointed to be an important element in the manifestations of cannabinoid-induced behavioral alterations. Indeed, extracellular levels of DA are enhanced upon THC administration [39][40]. Apparently, marijuana constituents, thus, modulate sleep in opposite directions.

4.4. *Cannabidiol and C-Fos.* Evaluation of c-Fos protein formation by CBD (**2**) in the dorsal striatum and *nucleus accumbens* of male *Wistar* rats was investigated to establish neuronal activation. After systemic administration of CBD (120 mg/kg), haloperidol (1 mg/kg), or clozapine (20 mg/kg), only haloperidol was able to increase the number of Fos immunoreactive neurons (FIr) in the dorsal striatum. In contrast, both haloperidol and CBD significantly increased FIr in the *nucleus accumbens.* Clozapine produced a barely significant increase in FIr. These results show that CBD is able to induce FIr in a limbic, but not in a motor-related, area [41].

**5. Cannabidiol: Selected Therapeutic Aspects.** – 5.1. *Neuroprotection*. Cannabinoids have been reported to provide neuroprotection in acute and chronic neurodegeneration [7]. *Lastres-Becker et al.* [42] examined whether they are also effective against the toxicity caused by 6-hydroxydopamine, both *in vivo* and *in vitro*, which may be relevant to *Parkinson*'s disease (PD). First, they evaluated whether the administration of cannabinoids *in vivo* reduces the neurodegeneration produced by a unilateral injection of 6-hydroxydopamine into the medial forebrain bundle. As expected, two weeks after application of this toxin, a significant depletion of dopamine contents and a reduction of tyrosine hydroxylase activity in the lesioned striatum were noted, accompanied by a reduction in the level of tyrosine-hydroxylase-mRNA in the *substantia nigra*. None of these events occurred in the contralateral structures. Daily

administration of THC (**1**) over these two weeks significantly lowered the magnitude of these reductions, whereas it failed to affect dopaminergic parameters in the contralateral structures. This effect appeared to be irreversible, since interruption of the daily administration of the cannabinoid after the two-week period did not lead to re-initiation of 6-hydroxydopamine-induced neurodegeneration. The same neuroprotective effect was also produced by CBD (**2**), which suggested that the anti-oxidant properties of both compounds, which are cannabinoid-receptor-independent, might be involved in these *in vivo* effects, although an alternative rationalization might be that the neuroprotection exerted by both compounds is due to their anti-inflammatory potential.

The same group [43] further explored this issue, with more selectivity for different elements of the cannabinoid-signaling system, using rats with unilateral lesions of nigrostriatal dopaminergic neurons caused by local application of 6-hydroxydopamine. Numerous cannabinoids were investigated. The authors also examined the timing for the effect of CBD to provide neuroprotection in this rat model of PD. They found that CBD, as expected, was able to recover 6-hydroxydopamine-induced DA depletion, when administered immediately after the lesion, but failed to do so when the treatment started one week later. In addition, the effect of CBD caused an upregulation of mRNA levels of Cu/Zn-superoxide dismutase, a key enzyme in endogenous defenses against oxidative stress. Their conclusion was that cannabinoids with anti-oxidant, cannabinoid-receptor-independent properties provide neuroprotection against the progressive degeneration of nigrostriatal dopaminergic neurons occurring in PD.

The possible neuroprotective mechanism of CBD, highlighting the importance of this compound to inhibit $\beta$-amyloid-induced neurodegeneration related to AD, has been studied in cultured rat pheocromocytoma PC12 cells [44]. First, following exposure of cells to $\beta$-amyloid peptide (1 μg/ml), a marked reduction in cell survival was observed. This effect was associated with increased ROS production and lipid peroxidation, as well as appearance of caspase-3 (a key enzyme in the apoptosis cell-signaling cascade), DNA fragmentation, and increased intracellular calcium. Treatment of the cells with CBD prior to the amyloid-peptide exposure significantly elevated cell survival, while it decreased ROS production, lipid peroxidation, caspase-3 levels, DNA fragmentation, and intracellular calcium. These results indicated that CBD exerted a combination of neuroprotective, anti-oxidative, and anti-apoptotic effects against $\beta$-amyloid-peptide toxicity, and that inhibition of caspase-3 appearance from its inactive precursor, pro-caspase-3, by CBD is involved in the signaling pathway for this neuroprotection.

In a further study [45][46], the same authors found that stimulation of differentiated PC12 cells with A$\beta$ (1–42) (1 μg/ml) for 36 h caused a significant increase of nitrite production, compared to non-stimulated cells. This production was inhibited in a concentration-dependent manner by both the non-selective iNOS inhibitor L-NAME (0.3–30 μM), and by the selective iNOS inhibitor SMT (0.3–30 μM). CBD ($10^{-6}$–$10^{-4}$ M) inhibited both nitrite production and iNOS protein expression induced by A$\beta$ (1–42). The CBD effect was mediated by inhibition of the phosphorylated form of the p38-MAP kinase and the transcription factor nuclear-factor-$\kappa$B (NF-$\kappa$B) activation in a concentration-dependent manner. These data are of considerable importance as a new route to inhibit $\beta$-amyloid-induced neurodegeneration, which is a typical sign of AD.

5.2. *Cerebral Ischemia*. The anti-oxidative and anti-inflammatory properties of CBD (**2**) led *Braida et al.* [47] to investigate its possible activity in preventing damage caused by cerebral ischemia. CBD (1.25–20 mg/kg) was administered to gerbils 5 min after a 10-min bilateral carotid-artery occlusion in freely-moving gerbils. Seven days after ischemia, it antagonized electroencephalographic flattening, with a dose-dependent bell-shaped curve, as seen in numerous other *in vivo* effects with CBD. The best neuroprotective effect was noted at 5 mg/kg. Histological examination showed complete survival of CA1 neurons in CBD-treated gerbils. These findings suggest a potential therapeutic role of CBD in cerebral ischemia. The mechanism of the protective action is not clear.

In a series of publications, *Fujiwara* and co-workers at Fukuoka University investigated the reduction of damage of cerebral infarction in mice upon treatment with CBD [12][48]. They noted that CBD significantly decreased infarct volume after cerebral-artery occlusion, which was not inhibited by a $CB_1$ antagonist, and was independent of hypothermia. However, as mentioned above, the CBD effect was blocked by a 5-HT1A antagonist. A recent publication by the same group [49] discloses that, while repeated treatment with THC (**1**) leads to the development of tolerance, this phenomenon is not observed with CBD (**2**).

5.3. *Type-1 Diabetes*. The therapeutic effects of CBD (**2**) in a model of rheumatoid arthritis, an autoimmune disease, led our group to investigate its action in a related disease, type-1 diabetes [50]. We found that CBD treatment of NOD mice[4]) before the development of the disease reduced the incidence from 86% in the non-treated control mice to 30% in CBD-treated mice. CBD Treatment also resulted in significant reduction of plasma levels of the pro-inflammatory cytokines, IFN-$\gamma$ and TNF-$\alpha$ . Th1-Associated cytokine production of *in vitro* activated T-cells and peritoneal macrophages was also significantly reduced in CBD-treated mice, whereas production of the Th2-associated cytokines IL-4 and IL-10 was increased, when compared to untreated control mice. Histological examination of the pancreatic islets of CBD-treated mice revealed significantly reduced insulitis. These data indicate that CBD can inhibit and delay destructive insulitis and inflammatory Th1-associated cytokine production in NOD mice, resulting in a decreased incidence of diabetes, possibly through an immunomodulatory mechanism shifting the immune response from Th1 to Th2 dominance.

In another publication recently submitted [51], we show that administration of CBD to female NOD mice, either in a latent diabetes stage (after 14 weeks) or with initial symptoms of diabetes (appearing up to 14 weeks), ameliorates the manifestations of the disease. Diabetes was diagnosed in only 32% of the mice in the CBD-treated group, compared to 100% in untreated groups. In addition, the level of the pro-inflammatory cytokine IL-12 produced by splenocytes was significantly reduced, whereas the level of the anti-inflammatory IL-10 was significantly elevated after CBD treatment. Histological examination of the pancreas of CBD-treated mice revealed more intact islets than in the controls. Our data strengthen the previous assumption that CBD, known to be safe in man, can possibly be used as a therapeutic agent for treatment of type-1 diabetes.

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[4]) Non-obese diabetes-prone mice that spontaneously develop diabetes.

The protective effects of CBD were also examined in streptozotocin-induced diabetic rats after one, two, or four weeks [52]. Retinal cell death, blood-retinal-barrier function, and oxidative stress were investigated by a variety of assays. Experimental diabetes induced significant increases in oxidative stress, retinal neuronal cell death, and vascular permeability. These effects were associated with increased levels of TNF-$\alpha$, vascular endothelial growth factor (VEGF), intercellular adhesion-molecule-1, and activation of p38-MAP kinase. CBD Treatment significantly reduced oxidative stress, decreased the levels of TNF-$\alpha$, VEGF, and intercellular adhesion-molecule-1, and prevented retinal cell death and vascular hyperpermeability in the diabetic retina. Consistent with these effects, CBD treatment also significantly inhibited p38-MAP kinase in the retina. These results demonstrate that CBD treatment reduces neurotoxicity, inflammation, and blood-retinal-barrier breakdown in diabetic animals through activities that may involve inhibition of p38-MAP kinase.

5.4. *Anti-Emetic and Antinausea Effects.* In a series of publications, in part reviewed previously [7], a Canadian group led by *Linda Parker* [53] reported that CBD (**2**) interferes with nausea in rats. THC (**1**) as well as CBD (**2**) were also found to potentiate extinction of a cocaine- and an amphetamine-induced conditioned place preference in rats. The cannabinoids did not affect learning or retrieval, and CBD was not hedonic on its own. These results are the first to show that both THC, which acts on both $CB_1$ and $CB_2$ receptors, and CBD, which does not bind to $CB_1$ or $CB_2$ receptors, potentiate the extinction of conditioned incentive learning [53].

As rats do not vomit, a better model for vomiting was sought. The house musk shrew (*Suncus murinus*) was found to be a suitable model, as it both vomits and expresses nausea [54]. In this model, inhibition of anticipatory nausea based on the emetic reactions of this mouse species was described [55]. Following three pairings of a novel distinctive contextual cue with the emetic effects of an injection of LiCl, the context acquired the potential to elicit conditioned retching in the absence of the toxin. The expression of this conditioned retching reaction was completely suppressed by pretreatment with each of the principal cannabinoids **1** and **2** found in marijuana, at a dose that did not suppress general activity. On the other hand, pretreatment with a dose of ondansetron (a 5-HT3 antagonist), that interferes with acute vomiting in this species, did not suppress the expression of conditioned retching during re-exposure to the Li-paired context. These results support anecdotal claims that marijuana, but not ondansetron, suppress the expression of anticipatory nausea.

5.5. *Anxiety.* Over the last two decades, numerous publications have examined the effect of CBD (**2**) in various anti-anxiety models (for a review, see [7]). A recent investigation using functional neuro-imaging has now given anatomical/physiological support to the previous positive reports [56]. Regional cerebral-blood flow was measured at rest using Single-Photon-Emission-Computed Tomography (SPECT) in ten healthy male volunteers, randomly divided into two groups of five subjects. The reactions of each subject were evaluated on two occasions, one week apart. In the first session, the subjects were given an oral dose of CBD (400 mg) or placebo, in a double-blind procedure. SPECT Images were acquired 90 min after drug ingestion. The Visual Analogue Mood Scale (VAMS) was applied to assess subjective states. In the second session, the same procedure was performed with the drug that had not been administered in the previous session. Comparisons of regional cerebral blood flow

were performed using suitable statistics. CBD significantly decreased subjective anxiety, and increased mental sedation, while placebo did not induce significant changes. Brain regions where anxiolytic effects of CBD were noted included the left amygdala–hippocampal complex, extending into the hypothalamus, and the left posterior cingulate gyrus. There was also greater activity with CBD than placebo in the left parahippocampal gyrus. These results confirm that CBD has anxiolytic properties, and that these effects are mediated by an action on limbic and paralimbic brain areas.

5.6. *Rheumatoid Arthritis*. The effect of CBD (**2**) was examined in two models of rheumatoid arthritis [10], both based on immunizing DBA/1 mice with type-II collagen (CII) in complete *Freund*'s adjuvant. The CII used was either bovine or murine, resulting in classical acute collagen-induced arthritis (CIA) or in chronic relapsing CIA, respectively. CBD was administered after onset of clinical symptoms. In both models, treatment effectively blocked the progression of arthritis. CBD was equally effective when administered i.p. or orally. The dose dependency showed a bell-shaped curve, with an optimal effect at 5 mg/kg per day (i.p.), or at 25 mg/kg per day (orally). Clinical improvement was associated with protection of the joints against severe damage. As mentioned above, draining lymph-node cells from CBD-treated mice showed a diminished CII-specific proliferation and IFN-$\gamma$ production, as well as a decreased release of TNF-$\alpha$ by synovial cells. These data show that CBD, through its combined immunosuppressive and anti-inflammatory actions, has a potent anti-arthritic effect in CIA.

In a later paper by the same group [57], using the same methods, related, though more potent, effects were found with a CBD-derived synthetic material, HU-320 ((−)-**6b**; see formula above). The authors concluded that the profound suppressive effects on cellular immune responses and on the production of pro-inflammatory mediators all indicated the usefulness of HU-320 as a novel non-psychoactive, synthetic, anti-inflammatory drug.

5.7. *Cancer*. The first documented experiments on the effects of CBD (**2**) on cancer cells were performed in the 1970s. The authors either found tumor-promoting or no effects at all. As these experiments were performed at extremely high doses (*e.g.*, 200 mg/kg [58]), it is unlikely that these observations are relevant to the current use of CBD in clinical trials or with the approved drug *Sativex*®, which contains CBD.

In the last years, there has been renewed interested in CBD as a potential anticancer drug. Although *Jacobsson et al.* did not find any significant effects on C6 rat glioma cells at CBD concentrations of up to 3 μM [59], it was later shown that this drug is able to inhibit the growth of two different human glioma cells in nude mice, both *in vitro* and *in vivo*. The $IC_{50}$ value for the two cell lines was *ca.* 25 μM, and apoptopic cell death was observed by two different assays. The cytotoxic effects could be blocked by a selective $CB_1$ antagonist, but only partially by a $CB_2$ antagonist. Surprisingly, pertussis toxin, which inactivates the known cannabinoid receptors, had no influence. The exact mechanism is not clear, as a vanilloid-receptor antagonist and inhibitors of ceramide generation, which are responsible for some of the cytotoxic effects of THC (**1**), failed to inhibit cell death [60].

These observations were further investigated, and it was found that CBD has an $IC_{50}$ value of 6–11 μM toward eight tumor cell lines, concentrations above 25 μM seemingly being cytotoxic on healthy cell lines. Depending on the cell type, pro-

apoptopic effects, cell-cycle arrest, and activation of caspase-3 were observed. As CBD inhibits anandamide uptake, it was assumed that the observed cytotoxicity might be caused by elevated anandamide levels, but more-efficient uptake inhibitors were not cytotoxic. The hypothesis that cell death was induced by oxidative stress was further supported by the cell-protective effects of the known anti-oxidants $\alpha$-tocopherol (vitamin E) and ascorbic acid (vitamin C), as well as by the observation that the formation of ROS depended both on intra- and extracellular calcium. The formation of ROS causes apoptosis, and when caspase-3 was inhibited, CBD was no longer effective. The potential use of CBD as an anticancer drug was further shown with two different tumor cell lines transplanted to nude mice. The tumors of the treated group were half as big as those of the untreated group, and both breast- and lung-cancer cells injected to paws showed approximately three times less metastatic invasion [61]. The effects of CBD on glioma cells was selective, as it did not cause apoptosis in primary glial cells [62].

CBD also caused apoptosis in human myeloblastic leukemia cells. At the highest tested concentration (8 μg/ml), 61% of the cells underwent apoptosis. This could be increased to 93%, when the cells were exposed to $\gamma$-radiation before CBD treatment. Interestingly, CBD, either with or without irradiation, did not cause apoptosis in healthy mononuclear cells [63]. This observation was confirmed by *McKallip et al.* [64], and the possible mechanisms were studied. Activation of caspases-3, -8, and -9, cleavage of poly(ADP-ribose) polymerase, and decreased levels of full-length Bid might present a cross-talk between the intrinsic and extrinsic pathways of apoptosis. As these effects could be blocked by the $CB_2$ antagonist SR144528, but not through a general inhibition of the Gi/Go pathway of $CB_2$ receptors by pertussis toxin, it might be postulated that SR144528 and CBD share an unknown binding site. However, another study showed no influence by $CB_2$ blockade on the cytotoxic effects of CBD [65]. In addition to these observed effects, the authors also showed increased expression of NAD(P)H oxidases. Induction of apoptosis and cell death was also present *in vivo* [64].

One of the resistance mechanisms of cancer cells is the overexpression of the ATP-binding cassette transporter, P-glycoprotein (P-gp), which effluxes several anticancer drugs. CBD at concentrations of up to 10 μM was not able to inhibit P-gp, but 1 μM CBD reduced the expression of P-gp by *ca.* 50%. In a cell line overexpressing P-gp, 10 μM CBD increased the cytotoxicity of vinblastine (a P-gp substrate) threefold [66]. These results are in contradiction to a second study [67], in which it was shown that CBD inhibits P-gp ($IC_{50} = 8.44$ μM), while THC (**1**), THC-COOH, and CBN did not significantly inhibit P-gp. Co-incubation of doxorubicin (a P-gp substrate and anticancer drug) with CBD showed a decreased efflux of doxorubicin, and increased intracellular concentrations. These different results might be due to the use of different cell lines expressing P-gp.

*R. M.* is grateful to the *National Institute on Drug Abuse*, U.S.A., for support over many decades. *R. M.* also kindly acknowledges a scholarship by the *Lesmuller Foundation*.

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*Received March 29, 2007*