Themed Issue: Cannabinoids

Review

Phytocannabinoids beyond the Cannabis plant – do they exist?

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It is intriguing that during human cultural evolution man has detected plant natural products that appear to target key protein receptors of important physiological systems rather selectively. Plants containing such secondary metabolites usually belong to unique chemotaxa, induce potent pharmacological effects and have typically been used for recreational and medicinal purposes or as poisons. Cannabis sativa L. has a long history as a medicinal plant and was fundamental in the discovery of the endocannabinoid system. The major psychoactive Cannabis constituent Δ⁹-tetrahydrocannabinol (Δ⁹-THC) potently activates the G-protein-coupled cannabinoid receptor CB₁ and also modulates the cannabinoid receptor CB₂. In the last few years, several other non-cannabinoid plant constituents have been reported to bind to and functionally interact with CB receptors. Moreover, certain plant natural products, from both Cannabis and other plants, also target other proteins of the endocannabinoid system, such as hydrolytic enzymes that control endocannabinoid levels. In this commentary we summarize and critically discuss recent findings.

Keywords: phytocannabinoid; cannabinoid; plant natural products; Cannabis; endocannabinoid system

Abbreviations: CB₁, type-1 cannabinoid receptor; CB₂, type-2 cannabinoid receptor; CP55940, (–)-cis-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-trans-4-(3-hydroxypropyl)cyclohexanol; Δ⁹-THC, Δ⁹-tetrahydrocannabinol; DIM, 3,3′-diindolylmethane; ECS, endocannabinoid system; FAAH, fatty acid amide hydrolase; FDA, US Food and Drug Administration; Gᵢ/o, G-protein alpha subunit; GPR55, orphan receptor G-protein-coupled receptor 55; MAGL, monoacylglycerol lipase; NAES, N-acylethanolamines; PPAR, peroxisome proliferator-activated protein; SR144528, (1S-endo)-5-(4-Chloro-3-methylphenyl)-1-((4-methylphenyl)methyl)-N-(1,3,3-trimethylbicyclo(2.2.1)hept-2-yl)-1H-pyrazole-3-carboxamide; SR141716A, N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide hydrochloride; Tmax, time to maximal concentration in plasma (pharmacokinetic parameter); TRPV1, transient receptor potential vanilloid-1 receptor; WIN55212-2, (R)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo-[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethylene

Today we perceive the endocannabinoid system (ECS) as a rather complex lipid signalling network in which different proteins play distinct roles in the control or in the modulation of numerous physiological and pathophysiological processes (Pertwee, 2005; Di Marzo, 2008). The ECS comprises classical cannabinoid receptors (CB₁ and CB₂), potentially also the orphan receptor GPR55, and arachidonic acid-derived ligands, which, however, also promiscuously target other receptors like, e.g. TRPV1 and PPAR-gamma (O’Sullivan, 2007; De Petrocellis and Di Marzo, 2010; Ross, 2009; Pertwee, 2010). Importantly, the enzymes degrading the endocannabinoids anandamide and 2-arachidonoyl glycerol, namely fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), have been shown to be promising therapeutic targets (Di Marzo, 2008). Finally, there appears to be an anandamide cellular reuptake mechanism that can be blocked by specific
inhibitors (Di Marzo, 2008). Both cannabinoid receptor agonists and antagonists have actual or potential therapeutic applications (Di Marzo, 2008; Oesch and Gertsch, 2009; Pertwee, 2009). Cannabinoids are defined as the terpenopheno- nolic constituents of Cannabis sativa L. and until recently, the phytlenperenoid Δ²-THC and some of its naturally occurring derivatives were the only plant natural products known to directly interact with cannabinoid receptors. However, in the last few years, several non-cannabinoid plant natural products have been reported to act as cannabinoid receptor ligands. This prompts us to define ‘phytocannabinoids’ as any plant-derived natural product capable of either directly interacting with cannabinoid receptors or sharing chemical similarity with cannabinoids or both. Direct cannabinoid receptor ligands are compounds that show high binding affinities (in the lower nM range) for cannabinoid receptors and exert discrete functional effects (i.e. agonism, neutral antagonism or inverse agonism). By contrast, indirect ligands target either key proteins within the ECS that regulate tissue levels of endocannabinoids or allosteric sites on the CB₁ receptor. Certain plant natural products, including some cannabinoids, possess at least some of these properties. Given the often high variability of molecular pharmacological data obtained in different laboratories and the distinct degrees of scrutiny of the experimental setup, molecular pharmacological data on natural products should always be interpreted with care (Gertsch, 2009). For example, the availability of CB receptor KO mice provides a powerful means of investigating the actual cannabimimetic nature of a particular compound in vivo. This commentary focuses on natural products from medicinal and dietary plants which have been reported to interact with the ECS.

**Fatty acid derivatives**

Despite the fact that N-acylthanolamines (NAEs) (Table 1) from plants do not interact with CB receptors (plants do not generally produce arachidonic acid, which is the acyl scaffold favoured for CB interaction) they have been shown to inhibit FAAH, thus leading to an increase in endocannabinoid tone. N-linoleoylthanolamide and N-oleoylthanolamide, which are found not only in chocolate (Theobroma cacao L.) but also other plants (Di Marzo et al., 1998), and the widespread NAE palmitoylethanolamide, inhibit anandamide breakdown (Maurelli et al., 1995; Di Tomaso et al., 1996). Certain N-alkylamides (alkamides) from Echinacea spp. (Table 2) have been shown to interact functionally with the human CB₁ receptor with low nM to μM K values (Gertsch et al., 2006). These N-isobutylamides selectively act at the CB₂ receptor over the CB₁ receptor, leading to an increase in intracellular calcium which could be blocked by the selective CB₂ receptor inverse agonist SR144528, but they do not modulate the Gₛₐ signalling pathway. Intriguingly, CB₂ receptor-binding N-alkylamides show similar anti-inflammatory effects as anandamide (e.g. inhibition of TNF-α) at low nM concentrations (Raduner et al., 2006). Certain Echinacea N-alkylamides inhibit anandamide reuptake in vitro (Chicca et al., 2009). Like anandamide, N-alkylamides also target PPAR-gamma (Spelman et al., 2009). Different Echinacea N-isobutylamides are orally bioavailable in rats and humans (Woelkart et al., 2008). The polycyclic polyene falcaminol, which is found in different plants of the Apiaceae family (e.g. in carrots) shows significant binding interactions with both cannabinoid receptors, but appears to selectively undergo an alkylation reaction with the CB₂ receptor (K value <1μM), leading to relatively potent inverse agonistic and pro-inflammatory effects in human skin (Leonti et al., 2010). Finally, it has been proposed that certain dietary fatty acids, which can also be found in plants, can modulate the ECS by influencing the availability of phospholipid biosynthetic precursors of endocannabinoids (Banni and Di Marzo, 2009).

**Terpenes**

The bicyclic sesquiterpene, β-caryophyllene (trans-isomer) (Table 2), which is a plant volatile very frequently found in plants, has been shown to selectively target the CB₂ receptor at nM concentrations (K = 155 nM) and to act as a full agonist (Gertsch et al., 2008). Remarkably, β-caryophyllene is also a major compound in Cannabis sativa L. essential oil. Thus, Cannabis produces two entirely different chemical scaffolds able to differentially target CB receptors. While studies on the pharmacokinetics of β-caryophyllene are still ongoing, it is already clear that this cyclobutane-ring containing terpene is readily bioavailable, and, unlike many polyphenolic natural products, is not metabolised immediately but shows a T_max >1 h after one single oral administration (J.G., unpublished data). Orally administered β-caryophyllene (<5 mg kg⁻¹) produces strong anti-inflammatory and analgesic effects in wild-type mice but not in CB₂ receptor knockout mice, which is a clear indication that it may be a functional CB₂ ligand. Ongoing studies show that β-caryophyllene is effective at reducing neuropathic pain in a CB₂ receptor-dependent manner (Zimmer et al., 2009). Therefore, the FDA approved food additive β-caryophyllene has the potential to become an attractive candidate for clinical trials targeting the CB₂ receptor (Gertsch, 2008). Interestingly, the diterpene salvinorin A from Salvia divinorum Epling & Jativa-M (Table 1) has been reported to be a selective high-affinity kappa-opioid receptor (KOP) agonist, but recent data also suggest that it may interact with a putative CB receptor/KOP heterodimer which may be formed during inflammatory conditions (Fichna et al., 2009). To date, binding experiments have shown that salvinorin A has very low affinity for homomeric cannabinoid receptors and does not inhibit endocannabinoid degradation (Capasso et al., 2008). Consequently, further research is needed to establish whether salvinorin A interacts with a putative cannabinoid/KOP heterodimeric receptor or whether the cannabimimetic effects reported are indirectly mediated via KOP. More recently, two naturally occurring quinonoid triterpe- noids, pristimerin (Table 1) and euphol, were found to inhibit MAGL with high potency (IC₅₀ = 93 nM and 315 nM respectively) through a reversible mechanism (King et al., 2009). As this class of triterpenes is relatively frequent in nature, it may not be unusual to find ‘indirect’ rather than ‘direct’ agonists of cannabinoid receptors among plant secondary metabolites. Several distinct triterpenes are known to modulate immune
<table>
<thead>
<tr>
<th>Structure</th>
<th>Name</th>
<th>Origin</th>
<th>CB receptor affinity</th>
<th>Function</th>
<th>In vivo efficacy</th>
<th>Other targets (ECS)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Structure" /></td>
<td>N-acylethanolamines</td>
<td>Widespread in plants</td>
<td>No affinity</td>
<td>FAAH inhibitors</td>
<td>Validated in CB₁ and CB₂ KO mice</td>
<td>GPR55</td>
<td>Maurelli et al., 1995; Di Tomaso et al., 1996; Di Marzo, 2008</td>
</tr>
<tr>
<td><img src="image2" alt="Structure" /></td>
<td>Salvinorin A</td>
<td>Salvia divinorum</td>
<td>Insignificant affinity to CB receptors</td>
<td>Indirect cannabimimetic effects at CB₁ (mechanism unknown)</td>
<td>No data</td>
<td>KOP agonist</td>
<td>Capasso et al., 2008; Fichna et al., 2009; Epling &amp; Jativa-M</td>
</tr>
<tr>
<td><img src="image3" alt="Structure" /></td>
<td>Pristimerin</td>
<td>Relatively widespread in the Celastraceae</td>
<td>No data</td>
<td>Potent reversible MAGL inhibitor (IC₅₀ value &lt;100 nM)</td>
<td>No data</td>
<td>No data</td>
<td>King et al., 2009</td>
</tr>
<tr>
<td><img src="image4" alt="Structure" /></td>
<td>Kaempferol</td>
<td>Widespread in plants</td>
<td>No affinity</td>
<td>FAAH inhibitor (IC₅₀ value &lt;1 µM)</td>
<td>No data</td>
<td>No data</td>
<td>Thors et al., 2007; 2008</td>
</tr>
<tr>
<td><img src="image5" alt="Structure" /></td>
<td>Trans-resveratrol</td>
<td>Relatively widespread in plants (e.g. Vitis vinifera L.)</td>
<td>Insignificant affinity</td>
<td>Insignificant effects</td>
<td>No data</td>
<td>No data</td>
<td>Prather et al., 2009</td>
</tr>
<tr>
<td><img src="image6" alt="Structure" /></td>
<td>Curcumin</td>
<td>Cucuma spp.</td>
<td>Insignificant affinity</td>
<td>Insignificant effects</td>
<td>No data</td>
<td>No data</td>
<td>Prather et al., 2009</td>
</tr>
<tr>
<td><img src="image7" alt="Structure" /></td>
<td>Epigallocatechin-3-O-gallate</td>
<td>Relatively widespread in plants (e.g. Camellia sinensis L.)</td>
<td>Insignificant affinity</td>
<td>No data</td>
<td>No data</td>
<td>No data</td>
<td>Korte et al., 2010</td>
</tr>
</tbody>
</table>

ECS, endocannabinoid system; FAAH, fatty acid amide hydrolase; MAGL, monoacylglycerol lipase.
**Table 2** Plant natural products that have been shown to interact directly with cannabinoid (CB) receptors

<table>
<thead>
<tr>
<th>Structure</th>
<th>Name</th>
<th>Origin</th>
<th>CB receptor affinity</th>
<th>Function</th>
<th>In vivo efficacy</th>
<th>Other targets (ECS)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Delta9-THC" /></td>
<td>Delta9-THC</td>
<td>Cannabis sativa L.</td>
<td>Non-selective CB&lt;sub&gt;1&lt;/sub&gt; and CB&lt;sub&gt;2&lt;/sub&gt; affinity (K&lt;sub&gt;i&lt;/sub&gt; values &lt;50 nM) (human)</td>
<td>Partial agonist G&lt;sub&gt;i/o&lt;/sub&gt; and CB&lt;sub&gt;2&lt;/sub&gt; receptor-selective inverse (covalent) analog</td>
<td>Validated in CB&lt;sub&gt;1&lt;/sub&gt; and CB&lt;sub&gt;2&lt;/sub&gt; KO mice</td>
<td>GPR55; PPARs; Different ion channels</td>
<td>Mechoulam, 1986; Pertwee, 2006</td>
</tr>
<tr>
<td><img src="image2" alt="N-alkylamide" /></td>
<td>N-alkylamide</td>
<td>Echinacea spp.</td>
<td>Selective CB&lt;sub&gt;2&lt;/sub&gt; affinity (K&lt;sub&gt;i&lt;/sub&gt; value &lt;100 nM) (human)</td>
<td>Partial agonist</td>
<td>No data</td>
<td>PPAR-γ inhibition of AEA transport; Partial FAAH inhibition</td>
<td>Raduner et al., 2006; Chicca et al., 2009</td>
</tr>
<tr>
<td><img src="image2" alt="N-alkylamide" /></td>
<td>N-alkylamide</td>
<td>Echinacea spp.</td>
<td>Selective CB&lt;sub&gt;2&lt;/sub&gt; affinity (K&lt;sub&gt;i&lt;/sub&gt; value &lt;100 nM) (human)</td>
<td>Partial agonist</td>
<td>No data</td>
<td>PPAR-γ inhibition of AEA transport; Partial FAAH inhibition</td>
<td>Raduner et al., 2006; Chicca et al., 2009</td>
</tr>
<tr>
<td><img src="image3" alt="Beta-caryophyllene" /></td>
<td>Beta-caryophyllene</td>
<td>Widespread in plants</td>
<td>Selective CB&lt;sub&gt;2&lt;/sub&gt; affinity (K&lt;sub&gt;i&lt;/sub&gt; value &lt;200 nM) (human)</td>
<td>Full agonist G&lt;sub&gt;i/o&lt;/sub&gt;</td>
<td>Validated in CB&lt;sub&gt;2&lt;/sub&gt; KO mice</td>
<td>No data</td>
<td>Gertsch et al., 2008</td>
</tr>
<tr>
<td><img src="image4" alt="Falcarnol" /></td>
<td>Falcarnol</td>
<td>Relatively widespread in Apiaceae (e.g. Daucus carota L.)</td>
<td>Non-selective CB&lt;sub&gt;1&lt;/sub&gt; affinity (K&lt;sub&gt;i&lt;/sub&gt; value &lt;1 μM) (human)</td>
<td>CB&lt;sub&gt;1&lt;/sub&gt; receptor-selective inverse (covalent) analog</td>
<td>No data</td>
<td>No data</td>
<td>Leonti et al., 2010</td>
</tr>
<tr>
<td><img src="image5" alt="Rutamarin" /></td>
<td>Rutamarin</td>
<td>Ruta graveolens L.</td>
<td>Selective CB&lt;sub&gt;2&lt;/sub&gt; affinity (K&lt;sub&gt;i&lt;/sub&gt; value &lt;10 μM) (human)</td>
<td>Partial agonist at CB&lt;sub&gt;2&lt;/sub&gt; receptor</td>
<td>No data</td>
<td>No data</td>
<td>Rollinger et al., 2009</td>
</tr>
<tr>
<td><img src="image6" alt="DIM" /></td>
<td>DIM</td>
<td>3,3-diindolylmethane metabolite from indole-3-carbinol</td>
<td>Selective CB&lt;sub&gt;2&lt;/sub&gt; affinity (K&lt;sub&gt;i&lt;/sub&gt; value &lt;1 μM) (human)</td>
<td>Partial agonist at CB&lt;sub&gt;2&lt;/sub&gt; receptor</td>
<td>No data</td>
<td>No data</td>
<td>Yin et al., 2009</td>
</tr>
</tbody>
</table>

Delta9-THC is shown as the major phytocannabinoid from Cannabis sativa L., but there are several other structurally related cannabinoids that interact with CB receptors.

Notes: ECS, endocannabinoid system; FAAH, fatty acid amide hydrolase; PPAR, peroxisome proliferator-activated protein.
functions through yet unknown mechanisms (Ríos, 2010) and it will thus be interesting to see in a more systematic study whether other similar triterpenoids are also able to inhibit MAGL.

Polyphenols

The dietary polyphenols trans-resveratrol and curcumin (Table 1) were reported to bind selectively to the human CB1 cannabinoid receptor with low nM Ki values (5.9 nM and 45 nM respectively) and to exert potent pharmacological effects in mice similar to those induced by the CB1 receptor inverse agonist rimonabant (Seely et al., 2009). Intrigued by this unexpected finding, our research groups independently measured the binding affinities of these compounds for CB1 and CB2 receptors in our laboratories. In our experiments, trans-resveratrol and curcumin only displaced [3H]CP55 940 from cannabinoid receptors at high μM concentrations, suggesting that they lack significant affinity for these receptors. Also polydatin, a glycosilated form of resveratrol, was inactive in these binding assays. Recently, the senior author of the original report retracted the paper (Prather et al., 2009). Hence, neither trans-resveratrol nor curcumin interact functionally with the CB1 receptor, despite the fact that these compounds appear to share the ability of the CB1 receptor inverse agonist, rimonabant, to induce weight loss in mice.

More recently, catechin-derivatives were shown to bind to human cannabinoid receptors rather non-selectively at high μM concentrations (Korte et al., 2010). Among these, epigallocatechin 3-gallate and (-)-epigallocatechin (Table 1) were reported to bind to the CB2 receptor with Ki values of 33.6 and 35.7 μM respectively. However, these Ki values may not be correct. For the calculation of the Ki values the Cheng-Prussof equation (Ki = IC50/(1+[S]/Kd)) was not applied correctly. The EC50 values used to calculate the Ki values were approximations as neither compound produced more than 60% radioligand displacement even at the highest concentration used. Catechins are very widespread plant secondary metabolites which may provide nutritional health benefits. The same group has recently reported similar CB1 and CB2 receptor Ki values for delphinidin and cyanidin, two hydrophilic anthocyanidins (Korte et al., 2009). In both reports, no functional data were shown. In our hands, flavonoid-type compounds (catechins, anthocyanidins, flavones) lead to negligible or very high Ki values, which likely reflect a nonspecific molecular denaturation of the protein surface rather than a functional binding interaction. Similar potentially artefactual effects would most likely be observed with other GPCRs.

Plant polyphenols, such as phenylpropanoids (e.g. epigallocatechin 3-O-gallate, curcumin, resveratrol) possess chemical scaffolds which at μM concentrations bind to protein targets in vitro with limited specificity. This is clearly reflected by numerous reports on protein binding interactions that such compounds undergo in the μM range (Anand et al., 2008; Bisht et al., 2009). At the macroscopic level, polyphenols (i.e. tannins) have been used to tan leather by denaturing of proteins, and at the microscopic level μM concentrations of polyphenols interact with multiple protein binding sites (via their hydroxyl groups) non-specifically and therefore such compounds score as frequent hitters in vitro. The great majority of established cannabinoid receptor ligands are highly lipophilic, which reflects the nature of the active site within cannabinoid receptors. Thus, hydrophilic polyphenols like catechins and anthocyanidins would clearly be atypical cannabinoid receptor ligands.

More interesting are findings that certain flavonoids inhibit fatty acid hydrolase (FAAH), which is the enzyme responsible for the breakdown of the endogenous CB receptor ligand anandamide (Thors et al., 2007; 2008). Both the isoflavonoid genistin and the flavonoids kaempferol (Table 1), 7-hydroxyflavone and 3,7-dihydroxyflavone have been shown to concentration-dependently inhibit anandamide hydrolysis in rat brain homogenates, albeit at relatively high concentrations (IC50 values between 2 and 10 μM). Nevertheless, the authors of these studies showed a preliminary structure-activity relationship with 7-hydroxyflavone being the most potent inhibitor (IC50 value <1 μM).

An abundant literature is devoted to mechanisms underlying the biological activity of plant polyphenols (Landis-Piwowar and Dou, 2008; Bisht et al., 2009). However, although most beneficial and potentially therapeutic effects of trans-resveratrol, curcumin, catechins and kaempferol-type flavonoids are typically detected in the low μM range in vitro, all such compounds show limited bioavailability and poor pharmacokinetics in vivo with reported plasma concentrations in the low nM range (DuPont et al., 2004; Garcea et al., 2004; Boocock et al., 2007).

Other plant natural products with binding affinity to the CB2 receptor

Other plant natural products have been shown to bind weakly to the CB2 receptor. These include the coumarin derivative rutamarin from the medicinal plant Ruta graveolens L. (Rollinger et al., 2009) and 3,3′-diindolylmethane (DIM) (Table 2), which is an anticarcinogenic metabolite generated by ingestion of indole-3-carbinol. Indole-3-carbinol is commonly found in Brassica vegetables. DIM has been shown to be a weak CB2 receptor partial agonist (Yin et al., 2009).

Conclusions

There is no doubt that phyto cannabinoids from Cannabis have greatly influenced research on the ECS and without the milestone discovery that Δ9-THC is the main psychoactive principle (reviewed in Mechoulam, 1986) many of the subsequent discoveries in the field of cannabinoid research would probably not have been made. Furthermore, with the development of therapeutic Cannabinoid extracts, as with Sativex™, this plant is also likely to provide new pharmaceutical applications in the future. The question remains as to why Cannabis sativa L. appears to be the only plant that produces a metabolite (Δ9-THC acid) that readily leads to its decarboxylation product Δ9-THC, which is the most potent phyto cannabinoid activator of the CB2 receptor. Interestingly enough, while nature may have been rather parsimonious in its provision of botanical secondary metabolites that activate the
CB₂ receptor, there is an increasing number of plant-derived natural products reported to target the CB₂ receptor to varying degrees. Flavonoids, which belong to natural polyphenols that readily interact with proteins, may target some of the proteins within the ECS, such as FAAH. However, no convincing evidence has been provided that polyphenols modulate cannabinoid receptors with significant potency. The finding that certain terpenes potently inhibit MAGL further adds to the repertoire of plant-produced ‘indirect’ cannabinoid receptor agonists. Although higher plants do not contain endocannabinoids and lack the classical G-protein-coupled cannabinoid receptors, they do express enzyme isoforms that resemble some of the enzymes known to be important in the processing of endocannabinoids (Shrestha et al., 2006). Plants produce fatty acid amides, some of which are able to inhibit the degradation of anandamide but do not generally bind to cannabinoid receptors with significant affinity to CB receptors (Gertsch et al., 2009). How scientific is the science in ethnopharmacology: historical perspectives and epistemological problems. J Ethnopharmacol 122: S177–S183.

Conflict of interest

The authors state no conflicts of interest.

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