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RESEARCH PAPER

Effects of cannabinoids and cannabinoid-enriched *Cannabis* extracts on TRP channels and endocannabinoid metabolic enzymes

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BACKGROUND AND PURPOSE

Cannabidiol (CBD) and Δ^9 -tetrahydrocannabinol (THC) interact with transient receptor potential (TRP) channels and enzymes of the endocannabinoid system.

EXPERIMENTAL APPROACH

The effects of 11 pure cannabinoids and botanical extracts [botanical drug substance (BDS)] from *Cannabis* varieties selected to contain a more abundant cannabinoid, on TRPV1, TRPV2, TRPM8, TRPA1, human recombinant diacylglycerol lipase α (DAGL α), rat brain fatty acid amide hydrolase (FAAH), COS cell monoacylglycerol lipase (MAGL), human recombinant N-acyl ethanolamine acid amide hydrolase (NAAA) and anandamide cellular uptake (ACU) by RBL-2H3 cells, were studied using fluorescence-based calcium assays in transfected cells and radiolabelled substrate-based enzymatic assays. Cannabinol (CBN), cannabichromene (CBC), the acids (CBDA, CBGA, THCA) and propyl homologues (CBDV, CBGV, THCV) of CBD, cannabigerol (CBG) and THC, and tetrahydrocannabivarin acid (THCVA) were also tested.

KEY RESULTS

CBD, CBG, CBGV and THCV stimulated and desensitized human TRPV1. CBC, CBD and CBN were potent rat TRPA1 agonists and desensitizers, but THCV-BDS was the most potent compound at this target. CBG-BDS and THCV-BDS were the most potent rat TRPM8 antagonists. All non-acid cannabinoids, except CBC and CBN, potently activated and desensitized rat TRPV2. CBDV and all the acids inhibited DAGL α . Some BDS, but not the pure compounds, inhibited MAGL. CBD was the only compound to inhibit FAAH, whereas the BDS of CBC > CBG > CBGV inhibited NAAA. CBC = CBG > CBD inhibited ACU, as did the BDS of THCVA, CBGV, CBDA and THCA, but the latter extracts were more potent inhibitors.

CONCLUSIONS AND IMPLICATIONS

These results are relevant to the analgesic, anti-inflammatory and anti-cancer effects of cannabinoids and *Cannabis* extracts.

LINKED ARTICLES

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Abbreviations

2-AG, 2-arachidonoylglycerol; 5-HT, 5-hydroxy-tryptamine; ACU, anandamide cellular uptake; AEA, anandamide; BDS, botanical drug substance; CB₁, cannabinoid receptor type-1; CB₂, cannabinoid receptor type-2; CBC, cannabichromene; CBD, cannabidiol; CBDA, cannabidiol acid; CBDV, propyl analogue of CBD or cannabidivarin; CBDVA, acid of the propyl analogue of CBD; CBG, cannabigerol; CBGA, cannabigerol acid; CBGV, propyl analogue of CBG or cannabigivarin; CBN, cannabitol; DAGL α , diacylglycerol lipase α ; EC, endocannabinoid; FAAH, fatty acid amide hydrolase; MAGL, monoacylglycerol lipase; NAAA, N-acylethanolamine acid amide hydrolase; THC, Δ^9 -tetrahydrocannabinol; THCA, THC acid; THCV, propyl analogue of THC or tetrahydrocannabivarin; THCVA, tetrahydrocannabivarin acid; TRP, transient receptor potential; TRPA1, TRP channel of ankyrin type 1; TRPM8, TRP channel of melastatin type-8; TRPV1, TRP channel of vanilloid type-1; TRPV2, TRP channel of vanilloid type-2

Introduction

The plant *Cannabis sativa* L. has been used for millennia as a medicinal agent for pain relief, as well as for recreational purposes. It contains over 100 well-characterized compounds derived from a diterpene structure, known as 'cannabinoids' (ElSohly and Slade, 2005; Mehmedic *et al.*, 2010). The large number of cannabinoids present in the plant – and the low naturally occurring levels of some of them – rendered it difficult to obtain sufficient quantities of such pure chemical entities in the past, thus explaining in part the slow progress of our understanding of the pharmacology of most of these compounds. Among them, (-)-*trans*- Δ^9 -tetrahydrocannabinol (THC), cannabidiol (CBD), cannabitol (CBN) and Δ^9 -tetrahydrocannabivarin (THCV) are the best characterized (Mechoulam and Shvo, 1963; Gaoni and Mechoulam, 1964). THC largely accounts for the psychoactive properties of marijuana (Mechoulam, 2000), and most of its effects are attributed to the activation of the cannabinoid CB₁ and CB₂ receptors, two G-protein-coupled receptors (GPCR) widely expressed in mammalian tissues (Matsuda *et al.*, 1990; Munro *et al.*, 1993; Van Sickle *et al.*, 2005; Gong *et al.*, 2006; Pertwee, 2008). The identification of CB₁ and CB₂ led to the isolation of their endogenous agonists, *N*-arachidonoyl-ethanolamine (anandamide, AEA) and 2-arachidonoylglycerol (2-AG) (Devane *et al.*, 1992; Mechoulam *et al.*, 1995; Sugiura *et al.*, 1995). Together with their receptors, these 'endocannabinoids' (EC) constitute the 'endocannabinoid system' (ECS), which also includes enzymes for EC biosynthesis and inactivation (Di Marzo *et al.*, 2005), the most studied of which are *N*-acylphosphatidylethanolamine-specific phospholipase D and diacylglycerol lipase α (DAGL α), for AEA and 2-AG biosynthesis, respectively, and fatty acid amide hydrolase (FAAH) and monoacyl glycerol lipase (MAGL), for AEA and 2-AG hydrolysis (Di Marzo *et al.*, 2005). Tissues produce several EC-like compounds with no direct activity at cannabinoid receptors, one of the most representative of which is *N*-palmitoylethanolamine (PEA), degraded by *N*-acylethanolamine acid amide hydrolase (NAAA) as well as FAAH (Ueda *et al.*, 2010).

Pharmacological evidence suggests that additional targets for cannabinoids and EC exist. Among the proposed non-CB₁/non-CB₂ receptors for cannabinoids, transient receptor potential (TRP) channels and some orphan GPCR (GPR55, GPR119) were suggested to bind EC or EC-related endogenous compounds (for a recent review see De Petrocellis and Di Marzo, 2010). Furthermore, less specific targets – ranging

from nuclear receptors to mitochondria and lipid rafts – were also proposed. The highly lipophilic nature of cannabinoids allows them to access intracellular sites of action, and non-CB₁/TRPV1-receptor-mediated increases of [Ca²⁺]_i by CBD have been observed in a variety of cell types (Ligresti *et al.*, 2006), including hippocampal neurones (Drysdale *et al.*, 2006). CBD actions on mitochondria and Ca²⁺ homeostasis provide a potential basis for CBD neuroprotective and anti-cancer properties (Massi *et al.*, 2004; García-Arencibia *et al.*, 2007; Iuvone *et al.*, 2009; Ryan *et al.*, 2009). However, CBD exhibits several other potentially beneficial properties, including strong anti-inflammatory actions (Costa *et al.*, 2004; 2007; Pertwee, 2004; Carrier *et al.*, 2006; Capasso *et al.*, 2008; Pan *et al.*, 2009). This compound has little or negligible affinity at CB₁ and CB₂ cannabinoid receptors (Pertwee, 2004), and might behave as a functional antagonist of CB receptor-mediated agonist effects in mouse brain and in membranes from cells transfected with human CB₂ receptors (Thomas *et al.*, 2007). Conversely, CBD potently increases the tissue levels of AEA by suppressing FAAH activity and/or by inhibiting the cellular uptake of AEA (Watanabe *et al.*, 1998; Bisogno *et al.*, 2001; Ligresti *et al.*, 2006), whereas when combined with THC it is able to increase CB₁ receptor expression (Hayakawa *et al.*, 2008). TRP channels of the ankyrin type-1 (TRPA1, the 'mustard oil' receptor), vanilloid-type 1 (TRPV1, the 'capsaicin receptor') and 2 (TRPV2) are activated by CBD (Bisogno *et al.*, 2001; De Petrocellis *et al.*, 2008; Qin *et al.*, 2008), whereas the TRP channel of melastatin type-8 (TRPM8, the 'menthol receptor') is antagonized by CBD (De Petrocellis *et al.*, 2008). Importantly, the activation, and subsequent desensitization of TRPV1 and TRPV2, which are deeply involved in transducing inflammatory and chronic pain at the peripheral and spinal level (Levine and Alessandri-Haber, 2007), underlie some of the anti-hyperalgesic actions of CBD (Costa *et al.*, 2004; 2007; Qin *et al.*, 2008). Possibly as a result of these effects, or of its more recently reported direct or indirect stimulant actions on adenosine (Carrier *et al.*, 2006) and 5-HT (5-HT_{1A}) (Russo *et al.*, 2005; Campos and Guimarães, 2008; Resstel *et al.*, 2009; Magen *et al.*, 2010; Yang *et al.*, 2010) receptors, CBD potentiates some therapeutic effects of THC (Varvel *et al.*, 2006; Marcu *et al.*, 2010), while counteracting some of its undesirable effects (i.e. sedation, psychotropic effects, tachycardia, hyperphagia, etc.), thus allowing the use of higher and more efficacious co-administered doses of THC (Russo and Guy, 2006). These observations led to the clinical development of Sativex®, the first cannabinoid botanical drug product, based on an

approximate 1:1 ratio of THC- and CBD-enriched *Cannabis* extracts, which was suggested to exhibit a higher therapeutic index than the corresponding pure cannabinoids (Russo and Guy, 2006).

Other cannabinoids, for example, CBN (a degradation product of THC) and cannabichromene (CBC), both demonstrate potent anti-inflammatory actions in the carrageenan paw oedema model of acute inflammation in rats (Sofia *et al.*, 1974; Turner and Elsohly, 1981; DeLong *et al.*, 2010). As some strains of *Cannabis* contain a considerable amount of CBC, its effects on THC actions were investigated (Hatoum *et al.*, 1981). Very recently it was shown that cannabigerol (CBG) activates α_2 -adrenoceptors and blocks G protein-coupled CB₁ and 5-HT_{1A} receptors (Cascio *et al.*, 2010). Finally, THCV, the propyl homologue of THC, is a CB₁ and CB₂ receptor antagonist (Thomas *et al.*, 2005) and a CB₂ agonist at higher doses, which attenuate inflammation and hyperalgesia in mice (Bolognini *et al.*, 2010). However, still very little is known of the mechanism of action of most of these and other 'minor' cannabinoids. Strains of cannabis (of defined genotype) expressing higher levels of these 'minor' cannabinoids as the principal cannabinoid have now been produced by GW Pharma Ltd.

In summary, non-psychoactive cannabinoids might contribute to several therapeutic benefits ascribed to *Cannabis*. On the other hand, the therapeutic use of THC is limited by its psychoactivity and potential for inducing dependence and tolerance, thus drawing further attention towards non-THC cannabinoids. The aim of the present study was to investigate the effects of 11 such compounds on TRPA1, TRPM8, TRPV1 and TRPV2 channels, as well as on DAGL α , FAAH, MAGL, NAAA and AEA cellular uptake (ACU). Apart from CBD, CBG, CBN and CBC, we also tested several cannabinoid propyl analogues and acids. For almost every cannabinoid tested, we also evaluated the activities of the corresponding cannabinoid extracts ['botanical drug substance' (BDS)]. The comparison between the effects observed with pure cannabinoids and the corresponding BDS might reveal the presence of potentially important synergistic effects that might be useful from a therapeutic point of view.

Methods

Compounds and extracts

CBC; CBD; CBG; CBN; cannabidiol acid (CBDA); cannabigerol acid (CBGA); cannabidivarin (CBDV); cannabigevarin (CBGV); cannabidivarin acid (CBDVA); Δ^9 -tetrahydrocannabinol (THC); Δ^9 -tetrahydrocannabinol acid (THCA); Δ^9 -tetrahydrocannabivarin (THCV); Δ^9 -tetrahydrocannabivarin acid (THCVA); and the corresponding BDS (extracts prepared from *Cannabis sativa* L. botanical raw material) were provided by GW Pharma Ltd (Salisbury, UK). The compounds were of at least 95% purity. The amount of each principal cannabinoid in the corresponding BDS varied between 40% and 70% (% w/w of extract) depending upon the BDS tested. The amount of each major cannabinoid in the BDS, expressed as a %, was used to calculate the amount of the BDS necessary to obtain the equimolar amount of the corresponding pure cannabinoid in the

various experiments. Thus, for example, if the amount of a given cannabinoid in a given BDS was 70%, the amount of BDS to be administered to cells to yield a given final concentration of the cannabinoid was calculated from the molecular weight of the cannabinoid and the amount in milligrams of the BDS (as if the BDS only contained the given cannabinoid) divided by 0.7. The chemical profile of minor cannabinoids present in each BDS was unique to each BDS, as was that of non-cannabinoid components. Thus, each BDS has a unique chemical profile ('chemical fingerprint').

TRP calcium assays

HEK-293 cells stably over-expressing recombinant rat TRPA1 rat TRPM8, rat TRPV2 or human TRPV1 were selected by G-418 (Geneticin; 600 $\mu\text{g}\cdot\text{mL}^{-1}$), grown on 100 mm diameter Petri dishes as monolayers in minimum essential medium supplemented with non-essential amino acids, 10% fetal bovine serum and 2 mM glutamine, and maintained under 5% CO₂ at 37°C. Stable expression of each channel was checked by quantitative-PCR. On the day of the experiment, the cells were loaded for 1 h at 25°C with the cytoplasmic calcium indicator Fluo-4AM (Invitrogen) 4 μM in dimethyl sulfoxide containing 0.02% Pluronic F-127 (Invitrogen, Carlsbad, CA, USA). After loading, cells were washed twice in Tyrode's buffer (145 mM NaCl, 2.5 mM KCl, 1.5 mM CaCl₂, 1.2 mM MgCl₂, 10 mM D-glucose and 10 mM HEPES, pH 7.4), resuspended in the same buffer, and transferred to the quartz cuvette of the spectrofluorimeter (ex λ = 488 nm; em λ = 516 nm) (Perkin-Elmer LS50B equipped with PTP-1 Fluorescence Peltier System; Perkin-Elmer Life and Analytical Sciences, Waltham, MA, USA) under continuous stirring. Experiments were carried by measuring cell fluorescence before and after the addition of various concentrations of phytocannabinoids. The values of the effect on [Ca²⁺]_i in wild-type (i.e. not transfected with any construct) HEK-293 cells were taken as baselines and subtracted from the values obtained from transfected cells. The potency of test compounds (EC₅₀ values) were determined as the concentration of test substances required to produce half-maximal increases in [Ca²⁺]_i. The efficacy of the agonists was determined by comparing their effect with the analogous effect observed with 4 μM ionomycin. The effects of the phytocannabinoids on TRPA1 are expressed as a percentage of the effect obtained with 100 μM allylisothiocyanate [mustard oil (MO)]. Antagonist/desensitizing behaviour was evaluated against capsaicin (0.1 μM) for TRPV1; icilin (0.25 μM) for TRPM8; MO (10 μM) for TRPA1; or lysophosphatidylcholine (LPC) (3 μM) for TRPV2 by adding the compounds in the quartz cuvette 5 min before stimulation of cells with agonists. Data are expressed as the concentration exerting a half-maximal inhibition of agonist effect (IC₅₀). The effect on [Ca²⁺]_i exerted by the agonist alone was taken as 100%. Some experiments were also carried out to antagonize the effects of CBDV and CBC on TRPA1-mediated elevation of [Ca²⁺]_i with AP18 (Petrus *et al.*, 2007) or HC030031 (McNamara *et al.*, 2007), which were added to cells 5 min before the agonists. All determinations were at least performed in triplicate. Dose-response curves were fitted by a sigmoidal regression with variable slope. Curve fitting and parameter estimation were performed with GraphPad Prism (GraphPad Software Inc., San Diego, CA, USA).

DAGL- α assay

Human recombinant DAGL- α was originally over-expressed with high efficiency in COS-7 cells and therefore these cells were used for this assay instead of HEK-293 cells, which, moreover, also express the native enzyme (Bisogno *et al.*, 2003). COS-7 cells, over-expressing DAGL- α , were homogenized in Tris-HCl buffer, pH 7. The homogenates were centrifuged at 4°C at 800 \times *g* (5 min) and then at 10 000 \times *g* (25 min). The 10 000 \times *g* membrane fraction was incubated in Tris-HCl 50 mM at pH 7.0 at 37°C for 20 min, with synthetic 1-[¹⁴C]-oleoyl-2-arachidonoyl-glycerol (1.0 mCi \cdot mmol⁻¹, 25 μ M) in the presence of vehicle or varying concentrations of test compounds. After the incubation, lipids were extracted with two volumes of CHCl₃/MeOH 2/1 (by volume). The extracts were lyophilized under vacuum and then purified by TLC on silica on polypropylene plates using CHCl₃/CH₃OH/NH₄OH (85/15/1 by volume) as the eluting system. Bands corresponding to [¹⁴C]-oleic acid were cut, and their radioactivity was counted with a β -counter.

MAGL assay

MAGL activity was measured using the cytosol derived from the 10 000 \times *g* fraction of homogenates from wild-type COS cells and synthetic 2 [³H]-arachidonoylglycerol; 100 μ g of protein and 10 μ M (10 000 cpm) [³H]-2-AG for each assay were incubated for 20 min at 37°C in the presence or absence of various concentrations of phytocannabinoids in Tris-HCl buffer, pH 7. [³H]-glycerol produced from the MAGL hydrolysis of [³H]-2-AG was measured by scintillation counting of the aqueous phase after extraction of the incubation mixture with two volumes of CHCl₃/CH₃OH (1:1 by volume) and used as a measure of enzyme activity.

FAAH assay

The effect of increasing concentrations of the compounds on the enzymatic hydrolysis of [¹⁴C]-AEA was studied using membranes prepared from rat brain. Membranes (70–100 μ g) were incubated in the presence or absence of varying concentrations of the compounds and [¹⁴C]-AEA (10 000 cpm, 2.5 μ M) in 50 mM Tris-HCl, pH 9, for 30 min at 37°C. [¹⁴C]-ethanolamine produced from [¹⁴C]-AEA hydrolysis was used to calculate FAAH activity and was measured by scintillation counting of the aqueous phase after extraction of the incubation mixture with two volumes of CHCl₃/CH₃OH (1:1 by volume). Data are expressed as the concentration exerting a half maximal inhibition (IC₅₀).

NAAA assays

HEK-293 cells in which the cDNA encoding the human enzyme was stably over-expressed (HEK-NAAA cells), were used for a specific enzymatic assay of NAAA as described previously (Saturnino *et al.*, 2010). Briefly, the 12 000 membrane fraction of homogenized cells was suspended in phosphate-buffered saline (pH 7.4) and subjected to two cycles of freezing and thawing and used in the assay. The enzyme preparation (50 μ g per sample) was incubated at 37°C for 30 min with 100 μ M [¹⁴C]-*N*-palmitoylethanolamine (10 000 cpm per sample) in a solution of citrate/sodium phosphate 50 mM (pH 5.2) and 0.1% Triton X-100, containing varying concentrations of the phytocannabinoids. The

reaction was terminated by the addition of chloroform/methanol (1:1 by volume) and quantification of [¹⁴C]-ethanolamine was carried out by using a β -counter (TRI-carb 2100TR; Perkin-Elmer) to quantify the [¹⁴C]-ethanolamine formed by the enzymatic activity.

ACU assay

The effect of compounds on ACU was analysed on rat basophilic leukaemia cells by using 2.5 μ M (10 000 cpm) [¹⁴C]-AEA. Cells were incubated with [¹⁴C]-AEA for 90 s at 37°C, in the presence or absence of varying concentrations of the compounds. Residual [¹⁴C]-AEA in the incubation medium after extraction with CHCl₃/CH₃OH 2:1 (by volume) was determined by scintillation counting of the lyophilized organic phase and was used as a measure of the AEA taken up by cells. Specific AEA uptake was determined by subtracting uptake at 4°C from uptake at 37°C. Data are expressed as the concentration exerting 50% inhibition of AEA uptake (IC₅₀) calculated by GraphPad software (Graph-Pad Software Inc., San Diego, CA, USA).

Results

Activity at TRPA1 channels

In Table 1, the efficacies and potencies of cannabinoids at eliciting a TRPA1-mediated increase in intracellular Ca²⁺ in HEK-293 cells over-expressing the channel are reported. HEK-293 cells were stably transfected with the plasmid pcDNA3 containing the rat recombinant TRPA1 cDNA, thus generating cells (denoted hereafter as 'TRPA1-HEK-293') that express high levels of TRPA1 transcript (De Petrocellis *et al.*, 2008). The compounds tested induced TRPA1-mediated Ca²⁺ elevation with efficacy comparable with that of allylisothiocyanate, the most potent being CBC (EC₅₀ = 90 nM) and CBD (EC₅₀ = 110 nM), in good agreement with the EC₅₀ values of 60 nM and 96 nM, respectively, previously published by our research group (De Petrocellis *et al.*, 2008). The rank order of potency of the pure compounds was as follows: CBC > CBD > CBN > CBDV > CBG > THCV > CBGV > THCA > CBDA > CBGA > THCVA. Therefore, none of the pure compounds was more potent than CBC, CBD and CBN as TRPA1 agonists. These compounds as well as CBDV and CBG were more potent than allylisothiocyanate, whereas the acid cannabinoids were the least active in this test. Except for THCA and CBN, all the pure cannabinoids also exhibited higher efficacy than allylisothiocyanate. Interestingly, THCV-BDS was the most potent (EC₅₀ = 70 nM), and other BDS (i.e. those of THCV, THCA, CBGV, CBDA, THCVA) were more potent (EC₅₀ = 0.07–5.8 μ M) than the corresponding pure compounds in this assay (Table 1). At high concentrations (>10 μ M), some compounds also caused an effect on [Ca²⁺]_i in cells that had not been transfected with any TRP construct (wild-type HEK-293 cells), and the values obtained from the latter cells were subtracted from those obtained in transfected cells. Importantly, all the compounds found here to activate TRPA1 also desensitized this channel to subsequent (5 min after addition of the cannabinoid) stimulation by MO allylisothiocyanates (Table 1). Finally, to provide conclusive evidence for the specificity of the effects, some compounds (i.e. CBDV and

Table 1

Efficacy and potency of pure cannabinoids and of most of their corresponding 'botanical drug substance' (BDS) as functional agonists at TRPA1 channels, and the potency of pure cannabinoids as desensitizers of TRPA1 to the effect of allyl isothiocyanate

Cannabinoid	Efficacy (% allyl isothiocyanate 100 μ M)	Potency shown as EC ₅₀ (μ M)	Desensitization (% allyl isothiocyanate 100 μ M) IC ₅₀ (μ M)
CBC	119.4 \pm 3.1	0.09 \pm 0.01	0.37 \pm 0.05
CBC-BDS	144.1 \pm 6.0	0.25 \pm 0.08	NT
CBD	115.9 \pm 4.6	0.11 \pm 0.05	0.16 \pm 0.05
CBD-BDS	163.6 \pm 11.9	0.38 \pm 0.15	NT
CBG	99.9 \pm 1.1	0.7 \pm 0.03	13.0 \pm 4.8
CBG-BDS	No effect	ND	NT
CBN	83.3 \pm 4.0	0.18 \pm 0.02	0.40 \pm 0.04
CBDA	113.0 \pm 11.0	5.3 \pm 1.5	4.92 \pm 0.09
CBDA-BDS	118.4 \pm 11.9	1.0 \pm 0.3	NT
CBGA	182.8 \pm 0.2	8.4 \pm 3.5	7.14 \pm 0.17
CBDV	105.0 \pm 0.7	0.42 \pm 0.01	1.29 \pm 0.38
CBDV-BDS	163.4 \pm 28.3	0.5 \pm 0.3	NT
CBGV	151.4 \pm 0.9	1.6 \pm 0.01	2.02 \pm 0.25
CBGV-BDS	106.4 \pm 4.0	0.8 \pm 0.1	NT
THC	117 \pm 12 ^a	0.23 \pm 0.03 ^a	NT
THCA	41.6 \pm 2.1	2.7 \pm 0.9	95.25 \pm 0.01
THCA-BDS	79.4 \pm 2.0	0.22 \pm 0.02	NT
THCV	243.0 \pm 16.5	1.5 \pm 0.6	3.07 \pm 0.24
THCV-BDS	197.6 \pm 7.9	0.07 \pm 0.02	NT
THCVA	170.2 \pm 15.9	16.4 \pm 2.4	13.14 \pm 0.85
THCVA-BDS	214.4 \pm 20.6	5.8 \pm 2.2	NT
Allyl isothiocyanate	100	1.4 \pm 0.01	NT

The effect of the compounds on the elevation of intracellular calcium was measured by fluorescence as described in Methods and was assessed in HEK-293 cells stably over-expressing the rat recombinant TRPA1 channel, and as a control, in wild-type HEK-293 cells. Data are means \pm SEM of at least $n = 3$ separate experiments in which various concentrations (1, 10, 100, 1000, 10 000 and 25 000 nM) of the pure compounds, or amounts of BDS estimated to contain equimolar concentrations of the major cannabinoid, were given to cells. Efficacy was calculated as % of the effect obtained with allyl isothiocyanate (100 μ M), the effect of which was ~30% of that of ionomycin (4 μ M). In the antagonism-desensitization experiments, the same concentrations of the pure compounds were given to cells 5 min prior to allyl isothiocyanate (100 μ M).

^aData are from De Petrocellis *et al.* 2008.

CBC, cannabichromene; CBD, cannabidiol; CBDA, cannabidiol acid; CBDV, propyl analogue of CBD or cannabidivarin; CBG, cannabigerol; CBGV, cannabigvarin; CBN, cannabinol; THC, Δ^9 -tetrahydrocannabinol; THCA, THC acid; THCV, propyl analogue of THC or tetrahydrocannabivarin; THCVA, tetrahydrocannabivarin acid; NT, not tested.

CBC) were also given to transfected cells that had been pre-incubated for 5 min with the TRPA1 antagonists AP18 (Petrus *et al.*, 2007) or HC030031 (McNamara *et al.*, 2007). The effect of CBDV (10 μ M) was reduced by AP18 (50 μ M) by 63.6 \pm 7.1% and by HC030031 (50 μ M) by 42.0 \pm 3.4%. These antagonists reduced the effect of MO isothiocyanate (10 μ M) by 51.0 \pm 8.2% and 51.5 \pm 5.2%, respectively. The effect of CBC (10 μ M) was reduced by AP18 (50 μ M) by 55.8 \pm 6.0%. AP18 (10 μ M) reduced the effects of CBC and MO isothiocyanate by 43.2 \pm 3.8 and 48.6 \pm 4.1% respectively (data are means \pm SD of $n = 3$ determinations).

Activity at TRPM8 channels

All the TRPM8 experiments were carried out at 22°C. None of the compounds tested activated TRPM8-mediated Ca²⁺ eleva-

tion in HEK-293 cells stably transfected with cDNA encoding the rat TRPM8 (denoted hereafter as 'TRPM8-HEK-293'), and overexpressing high levels of TRPM8 transcript (De Petrocellis *et al.*, 2007). Instead, when the compounds were given to cells 5 min before the known TRPM8 agonist, icilin (0.25 μ M), they all, with the exception of CBC, antagonized the Ca²⁺ elevation response. As shown previously, icilin dose dependently elevated intracellular Ca²⁺ in TRPM8-HEK-293 cells, but not in non-transfected cells, with an EC₅₀ of 0.19 \pm 0.03 μ M (De Petrocellis *et al.*, 2007) (Table 2). The rank order of potency of the pure compounds against icilin was: CBD > CBG > THCA > CBN > THCV > CBDV > CBGA > THCVA > CBGV > CBDA (IC₅₀ = 0.06–4.8 μ M), but also some BDS (THCV, CBG, THCA) were very potent (IC₅₀ < 0.1 μ M) at antagonizing TRPM8 (Table 2). A 'CBG-free' CBG-BDS was

Table 2

Potency of pure cannabinoids and of most of their corresponding 'botanical drug substance' (BDS) as functional antagonists at TRPM8 channels

Cannabinoid	IC ₅₀ μ M (against icilin 0.25 μ M)
CBC	40.7 \pm 0.6
CBC-BDS	13.0 \pm 1.1
CBD	0.06 \pm 0.01
CBD-BDS	1.29 \pm 0.04
CBG	0.16 \pm 0.02
CBG-BDS	0.034 \pm 0.003
CBN	0.21 \pm 0.05
CBDA	4.78 \pm 0.01
CBDA-BDS	1.2 \pm 0.1
CBGA	1.31 \pm 0.09
CBDV	0.90 \pm 0.01
CBDV-BDS	1.2 \pm 0.05
CBGV	1.71 \pm 0.04
CBGV-BDS	0.95 \pm 0.03
THC	0.16 \pm 0.01 ^a
THCA	0.15 \pm 0.02
THCA-BDS	0.056 \pm 0.0026
THCV	0.87 \pm 0.006
THCV-BDS	0.02 \pm 0.006
THCVA	1.33 \pm 0.019
THCVA-BDS	0.45 \pm 0.057

The effect of the compounds on the elevation of intracellular calcium was measured by fluorescence as described in Methods and was assessed in HEK-293 cells stably over-expressing the rat recombinant TRPM8 channel, and as a control, in wild-type HEK-293 cells. Data are means \pm SEM of at least $n = 3$ separate experiments in which various concentrations (1, 10, 100, 1000, 10 000 and 25 000 nM) of the pure compounds, or amounts of BDS estimated to contain equimolar concentrations of the major cannabinoid, were given to cells 5 min prior to icilin (0.25 μ M). None of the compounds exerted any significant TRPM8-mediated effect on intracellular calcium per se (not shown).

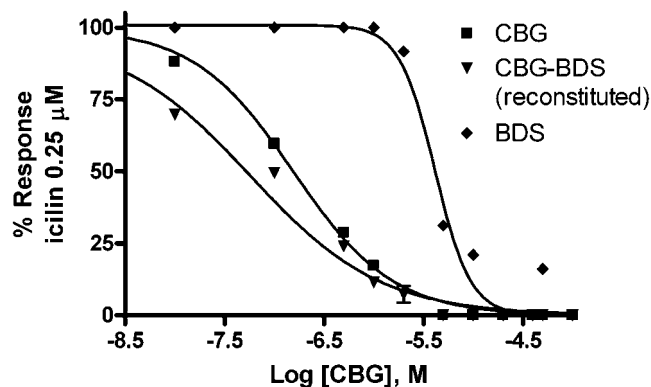
^aData are from De Petrocellis *et al.*, 2008.

CBC, cannabichromene; CBD, cannabidiol; CBDA, cannabidiol acid; CBDV, propyl analogue of CBD or cannabidivarin; CBG, cannabigerol; CBGV, cannabigivarin; CBN, cannabinol; THC, Δ^9 -tetrahydrocannabinol; THCA, THC acid; THCV, propyl analogue of THC or tetrahydrocannabivarin; THCVA, tetrahydrocannabivarin acid.

found to be inactive *per se*, but when added to pure CBG, the activity of CBG-BDS was restored and this compound was significantly more potent and efficacious at antagonizing TRPM8 than pure CBG (Figure 1), thus pointing to a synergistic effect between this cannabinoid and some of the components of its corresponding *Cannabis* extract.

Activity at TRPV1 channels

Given the previously reported effects of some cannabinoids on TRPV1 (Bisogno *et al.*, 2001; Ligresti *et al.*, 2006), it was

**Figure 1**

Inhibition of TRPM8-mediated elevation of intracellular calcium by cannabigerol (CBG), a CBG-botanical drug substance rendered free of CBG [denoted as botanical extract (BDS)] and the reconstituted CBG-BDS (denoted as CBG-BDS reconstituted). Experiments were carried out in HEK-293 cells stably over-expressing the rat recombinant TRPM8 channel and are means of at least $n = 3$ separate experiments. 'CBG-BDS reconstituted' was obtained by mixing the amount of CBG present in a given amount of CBG-BDS and the remaining amount of the 'CBG-free' BDS. The concentrations tested of 'CBG-BDS reconstituted' were calculated to contain equimolar concentrations of CBG as in pure CBG. The potency of 'CBG-BDS reconstituted' was slightly lower than that shown in Table 2 for CBG-BDS but still higher than that of CBG. SEM are not shown for the sake of clarity and were never higher than 10% of the means.

interesting to assess whether those compounds belonging to this class that had not been tested before on TRPV1 can interact with this target. Apart from the previously reported stimulating effects of CBD and CBG on TRPV1 (Bisogno *et al.*, 2001; Ligresti *et al.*, 2006), we found here that THCV and CBGV, but much less so their acid analogues, also stimulated human TRPV1 ($EC_{50} = 1.0$ – 2.0μ M), with the BDS being less efficacious than the corresponding cannabinoid, except for THCV-BDS (0.2 μ M) (Table 3, Figure 2). Many compounds exhibited very weak efficacy at TRPV1 receptors, often hardly measurable. Therefore, the EC_{50} values calculated for these compounds may have little pharmacological meaning. Nevertheless, several compounds also desensitized TRPV1 to its subsequent (5 min after addition of the cannabinoid) stimulation by capsaicin (Table 3).

Activity at TRPV2 channels

The pure compounds available were also tested on TRPV2, based on the previously reported agonistic effect of CBD on this channel (Qin *et al.*, 2008). We stably transfected HEK-293 cells with the plasmid pcDNA3 containing the rat recombinant TRPV2 cDNA, thus generating cells (denoted hereafter as 'TRPV2-HEK-293') that, as assessed by real-time PCR, express high levels of TRPV2 transcript (data not shown). This transcript was absent in HEK-293 cells that had not been transfected with the pcDNA3 plasmid and were treated with only Lipofectamine (data not shown). Using our fluorometric test, we showed that rat TRPV2-HEK293 cells exhibit a sharp increase in intracellular $[Ca^{2+}]_i$ upon application of LPC or 2-aminoethoxydiphenyl borate, two well-established TRPV2

Table 3

Efficacy and potency of pure cannabinoids and of most of their corresponding 'botanical drug substance' (BDS) as functional agonists at TRPV1 channels, and the potency of pure cannabinoids as desensitizers of TRPV1 to the effect of capsaicin

Cannabinoid	Efficacy (% ionomycin 4 μM)	Potency shown as EC_{50} (μM)	Desensitization (% capsaicin 0.1 μM) IC_{50} (μM)
CBC	<10	24.2 \pm 3.1	>50
CBC-BDS	29.1 \pm 0.9	8.6 \pm 1.2	NT
CBD	44.7 \pm 0.02	1.0 \pm 0.1	0.6 \pm 0.05
CBD-BDS	59.9 \pm 1.4	1.2 \pm 0.4	NT
CBG	33.8 \pm 2.3	1.3 \pm 0.5	2.6 \pm 0.2
CBG-BDS	23.0 \pm 0.1	2.7 \pm 0.1	NT
CBN	<10	6.2 \pm 3.7	81.7 \pm 9.0
CBDA	<10	19.7 \pm 3.9	89.1 \pm 0.3
CBDA-BDS	15.2 \pm 0.01	10.3 \pm 0.02	NT
CBGA	72.8 \pm 2.0	21.0 \pm 1.25	20.6 \pm 0.5
CBDV	21.4 \pm 0.6	3.6 \pm 0.7	10.0 \pm 0.5
CBDV-BDS	48.3 \pm 1.1	4.5 \pm 0.6	NT
CBGV	58.8 \pm 0.9	2.0 \pm 0.1	2.3 \pm 0.3
CBGV-BDS	67.5 \pm 1.8	3.9 \pm 0.2	NT
THC	<10	ND	NT
THCA	<10	ND	19.2 \pm 5.3
THCA-BDS	<10	17.3 \pm 0.1	NT
THCV	68.0 \pm 1.6	1.5 \pm 0.2	1.3 \pm 0.1
THCV-BDS	48.9 \pm 1.5	0.2 \pm 0.04	NT
THCVA	20.0 \pm 0.5	25.6 \pm 1.1	>50
THCVA-BDS	17.0 \pm 1.4	29.0 \pm 7.2	NT

The effect of the compounds on the elevation of intracellular calcium was measured by fluorescence as described in Methods and was assessed in HEK-293 cells stably overexpressing the human recombinant TRPV1 channel, and as a control, in wild-type HEK-293 cells. Data are means \pm SEM of at least $n = 3$ separate experiments in which various concentrations (1, 10, 100, 1000, 10 000 and 25 000 nM) of the pure compounds, or amounts of BDS estimated to contain equimolar concentrations of the major cannabinoid, were given to cells. Efficacy was calculated as % of the effect obtained with ionomycin (4 μM). Capsaicin efficacy in this test is ~75% of the effect of ionomycin. In the antagonism-desensitization experiments, the same concentrations of the pure compounds were given to cells 5 min prior to capsaicin (0.1 μM). CBC, cannabichromene; CBD, cannabidiol; CBDA, cannabidiol acid; CBDV, propyl analogue of CBD or cannabidivarin; CBG, cannabigerol; CBGV, cannabigivarin; CBN, cannabinol; THC, Δ^9 -tetrahydrocannabinol; THCA, THC acid; THCV, propyl analogue of THC or tetrahydrocannabivarin; THCVA, tetrahydrocannabivarin acid; ND, not determined; NT, not tested.

activators (Neeper *et al.*, 2007; Monet *et al.*, 2009). Using this test, we determined the concentration for half-maximal activation to be 3.4 \pm 0.025 μM for LPC (Table 4, Figure 3). Analogous to the findings of Qin *et al.* (2008), THC and CBD, as well as several of the pure cannabinoids that were tested, increased $[\text{Ca}^{2+}]_i$ in TRPV2-HEK-293 cells. The EC_{50} values are shown in Table 4, and the rank of potency was THC > CBD > CBGV > CBG > THCV > CBDV > CBN. Conversely, all the carboxylic acid cannabinoids and CBC were inactive or showed a very weak effect, as in the case of THCA, 18.4 \pm 0.9 (3.0 \pm 0.02) (Table 4). However, a 5 min preincubation of TRPV2-HEK-293 cells with CBGV, THC, CBG, CBD and THCV dose dependently abolished the elevation of $[\text{Ca}^{2+}]_i$ induced by LPC 3 μM (Table 4). Importantly, the order of potency for desensitization did not merely reflect the rank order of potency for channel activation.

Activity at 2-AG biosynthesizing and degrading enzymes

Two enzymes involved in the biosynthesis of 2-AG from diacylglycerol precursors were cloned and named *sn*-1-specific DAGL α and β . DAGL α was found in the adult brain to be mostly localized in post-synaptic neurones (Bisogno *et al.*, 2003). An MAGL inactive on AEA has been suggested to act as a major 2-AG degrading enzyme (Dinh *et al.*, 2002). Both DAGL and MAGL are members of the large lipase-3 family of Ser hydrolases. Several cannabinoids were screened here as possible DAGL α and MAGL inhibitors. The inhibitory effect of compounds on DAGL α was tested in COS cells overexpressing human DAGL α , the most abundant of the two DAGLs in the adult brain (Bisogno *et al.*, 2003); wild-type (i.e. non-transfected) COS cells also exhibited DAGL activity but

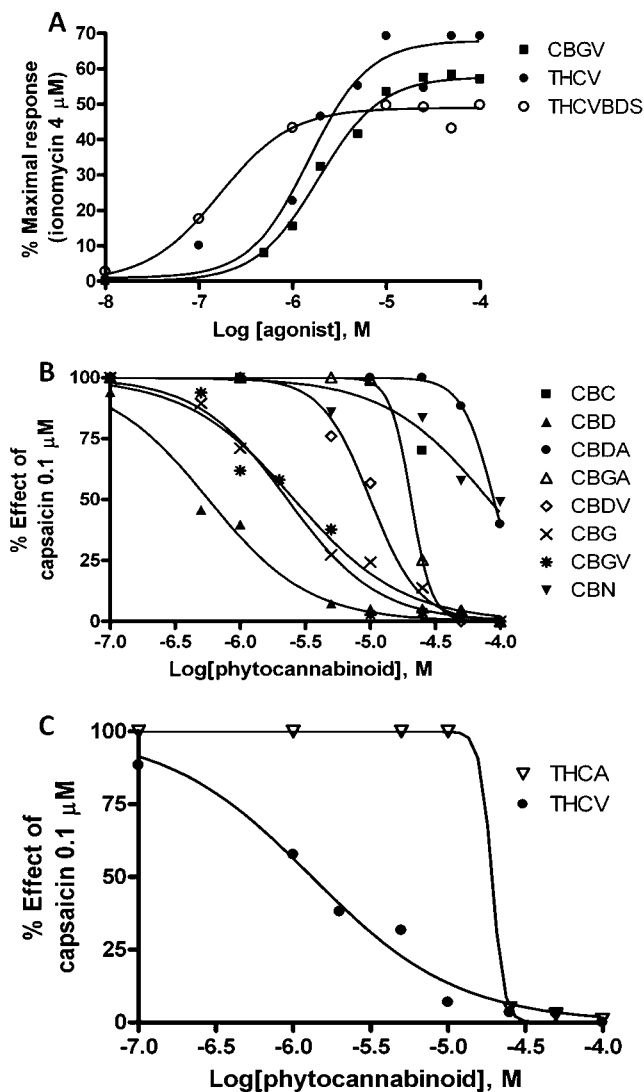


Figure 2

(A) Dose-dependent effects of cannabigvarin (CBGV), Δ^9 -tetrahydrocannabivarin (THCV) and THCv-botanical drug substance (THCV-BDS) on TRPV1-mediated elevation of intracellular calcium in HEK-293 cells stably over-expressing the human recombinant TRP channel of vanilloid type-1 (TRPV1) channel (HEK-293-TRPV1 cells). (B) Dose-dependent inhibitory effects of a 5 min pre-incubation of HEK-293-TRPV1 cells with several pure cannabinoids on the TRPV1-mediated elevation of intracellular calcium induced by capsaicin (0.1 μ M). (C) Dose-dependent inhibitory effects of a 5 min pre-incubation of HEK-293-TRPV1 cells with THCv and THCA on the TRPV1-mediated elevation of intracellular calcium induced by capsaicin (0.1 μ M). Data are means of at least $n = 3$ separate experiments. SEM are not shown for the sake of clarity and were never higher than 10% of the means. CBC, cannabichromene; CBD, cannabidiol; CBG, cannabigerol; CBGV, cannabigvarin; CBN, cannabinol; THC, Δ^9 -tetrahydrocannabinol; THCv, propyl analogue of THC or tetrahydrocannabivarin.

this was significantly lower than the activity of cells transfected with human DAGL α cDNA and was subtracted from the latter when calculating the IC_{50} values of the inhibitors (Bisogno *et al.*, 2006). As reported in Table 5, CBDV ($IC_{50} =$

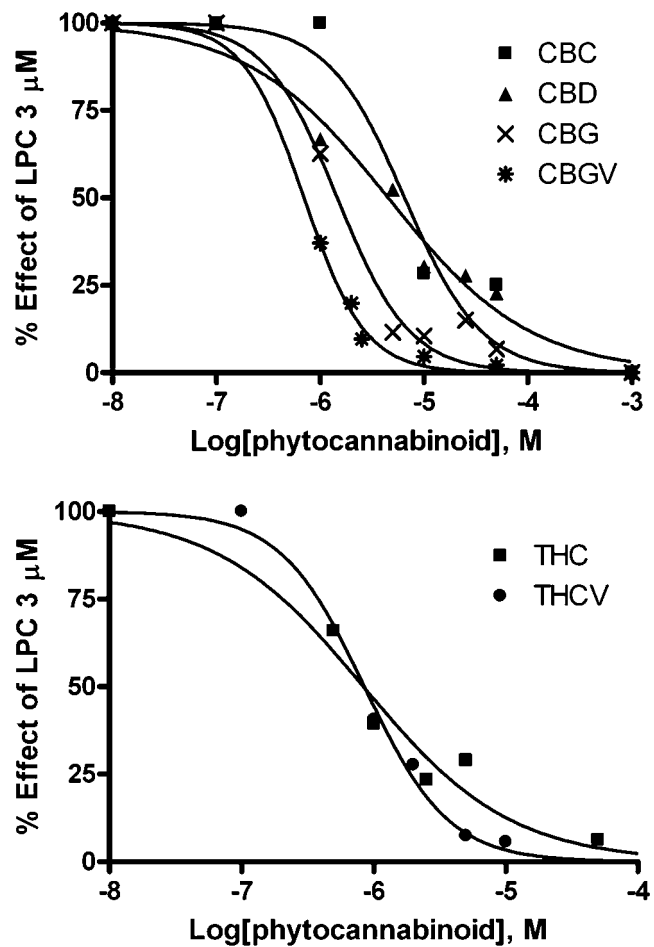


Figure 3

Dose-dependent effects of several pure cannabinoids on lysophosphatidylcholine (LPC, 3 μ M)-induced, TRP channel of vanilloid type-2 (TRPV2)-mediated elevation of intracellular calcium in HEK-293 cells stably over-expressing the rat recombinant TRPV2 channel. Compounds were given to cells 5 min prior to LPC. Data are means of at least $n = 3$ separate experiments. SEM are not shown for the sake of clarity and were never higher than 10% of the means. CBC, cannabichromene; CBD, cannabidiol; CBG, cannabigerol; CBGV, cannabigvarin; THC, Δ^9 -tetrahydrocannabinol; THCv, propyl analogue of THC or tetrahydrocannabivarin.

16.6 μ M), and all the acids tested, exhibited inhibitory effects on DAGL α ($IC_{50} = 19.4\text{--}65.7 \mu$ M). Of all the BDS tested, DAGL α inhibitory activity was found only in the CBDA-BDS and THCA-BDS. Because, of the pure compounds, only CBDV and all the acids inhibited DAGL α , we reasoned that the acid of CBDV might be even more potent. Therefore, we prepared a few milligrams of CBDVA and tested it only in this assay. However, this compound was not more potent than CBDV and CBDA (Table 5).

The effect of the compounds on MAGL was studied in cytoplasmic fractions of COS cells by using [3 H]-2-AG as a substrate, as we had previously reported that the enzyme was abundantly expressed in this preparation (Bisogno *et al.*, 2006). None of the pure compounds exhibited any significant inhibition up to 50 μ M (Table 6), whereas some BDS

Table 4

Efficacy and potency of pure cannabinoids as functional agonists at TRPV2 channels, and their potency as desensitizers of TRPV2 to the effect of lysophosphatidylcholine (LPC)

Cannabinoid	Efficacy (% ionomycin 4 μ M)	Potency shown as EC ₅₀ (μ M)	Desensitization (% LPC 3 μ M) IC ₅₀ (μ M)
CBC	<10	ND	6.5 \pm 1.6
CBD	40.5 \pm 1.6	1.25 \pm 0.23	4.5 \pm 0.7
CBG	73.6 \pm 1.2	1.72 \pm 0.08	1.5 \pm 0.2
CBN	39.9 \pm 2.1	19.0 \pm 3.7	15.7 \pm 2.1
CBDA	<10	ND	114.0 \pm 18.0
CBGA	<10	ND	87.3 \pm 1.2
CBDV	49.9 \pm 0.9	7.3 \pm 0.4	31.1 \pm 0.2
CBGV	75.4 \pm 2.4	1.41 \pm 0.36	0.7 \pm 0.06
THC	53.0 \pm 1.4	0.65 \pm 0.05	0.8 \pm 0.1
THCA	68.2 \pm 1.0	18.4 \pm 0.9	9.8 \pm 2.6
THCV	73.8 \pm 1.0	4.11 \pm 0.11	0.8 \pm 0.5
THCVA	<10	ND	104.3 \pm 9.9
LPC	91.71 \pm 0.47	3.37 \pm 0.025	NT

The effect of the compounds on the elevation of intracellular calcium was measured by fluorescence as described in Methods and was assessed in HEK-293 cells stably overexpressing the rat recombinant TRPV2 channel, and as a control, in wild-type HEK-293 cells. Data are means \pm SEM of at least $n = 3$ separate experiments in which various concentrations (1, 10, 100, 1000, 10 000 and 25 000 nM) of the pure compounds were given to cells. Efficacy was calculated as % of the effect obtained with ionomycin (4 μ M). In the antagonism-desensitization experiments, the same concentrations of the pure compounds were given to cells 5 min prior to LPC (3 μ M).

CBC, cannabichromene; CBD, cannabidiol; CBDA, cannabidiol acid; CBDV, propyl analogue of CBD or cannabidivarin; CBG, cannabigerol; CBGV, cannabigivarin; CBN, cannabinol; THC, Δ^9 -tetrahydrocannabinol; THCA, THC acid; THCV, propyl analogue of THC or tetrahydrocannabinol; THCVA, tetrahydrocannabivarin acid; ND, not determined; NT, not tested.

exhibited significant MAGL inhibitory efficacy (IC₅₀ = 6.2–29.6 μ M), CBG-BDS and THCA-BDS being the most potent (Table 6). Indeed, a 'CBG-free' CBG-BDS was found to be weakly active *per se*, but, when added to pure CBG, the activity of CBG-BDS was restored and this reconstituted extract was significantly more potent and efficacious at inhibiting MAGL than pure CBG (Figure 4).

Activity at AEA and PEA degrading enzymes

AEA and PEA are inactivated by enzymatic hydrolysis catalyzed by amidases, the best characterized of which is the FAAH (Cravatt *et al.*, 1996). All compounds were therefore evaluated in assays of FAAH using [¹⁴C]-AEA hydrolysis by rat brain membranes (which express FAAH as the only AEA hydrolyzing enzyme). The effects are shown in Table 7. It is noteworthy that CBD was the only compound that inhibited FAAH, this effect having been already reported previously (Watanabe *et al.*, 1998).

PEA inactivation to palmitic acid and ethanolamine is catalyzed by FAAH and, more selectively, NAAA (Ueda *et al.*, 2001). The screening of different cannabinoids allowed us to identify three BDS preparations that inhibited NAAA: CBC-BDS > CBG-BDS > CBGV-BDS (IC₅₀ = 14.2–24.4 μ M). The only pure compound with appreciable inhibitory activity was CBDA (IC₅₀ = 23.0 μ M) (Table 8). All these compounds were selective for NAAA, and were significantly much less active or inactive at FAAH, with IC₅₀ values always higher than 50 μ M (Table 7).

The extent and duration of AEA actions are determined by its extracellular concentration, controlled in turn by the rate of AEA synthesis and degradation. As the hydrolytic enzymes are intracellularly located, an efficient transport system allowing for the rapid trafficking of AEA through these topographically separated sites was proposed. Although this putative 'carrier' has yet to be cloned, some indirect biochemical and pharmacological evidence suggests its existence (Di Marzo *et al.*, 1994; Ligresti *et al.*, 2004; 2010). The 21 cannabinoid preparations were therefore evaluated for their activity on [¹⁴C]-AEA uptake by intact RBL-2H3 cells. Eleven were found to be active (Table 9), and interestingly none of the most potent preparations inhibited FAAH (Table 7). CBC = CBG > CBD inhibited AEA uptake most efficaciously and potently (IC₅₀ = 11.3–25.3 μ M), but some BDS (i.e. those of THCVA, CBGV, CBDA, THCA) were even more potent (IC₅₀ = 5.8–12.5 μ M) than the corresponding pure compounds in this assay (Table 9).

Discussion

The results reported here can be summarized as follows:

- Cannabinoids can activate TRPA1, TRPV1 and TRPV2, and functionally antagonize TRPM8 receptors in a manner dependent on their chemical structure – importantly, the structural prerequisites necessary to interact with the

Table 5

Efficacy and potency of pure cannabinoids and of most of their corresponding 'botanical drug substance' (BDS) as inhibitors of human recombinant DAGL α

Compounds	IC ₅₀ (μ M)	Maximum concentration tested (% inhibition)
CBC	>100 μ M	100 μ M 39.5 \pm 2.9
CBC-BDS	>100 μ M	100 μ M 36.5 \pm 7.1
CBD	>100 μ M	100 μ M 0
CBD-BDS	>50 μ M	50 μ M 8.5 \pm 3.1
CBG	>100 μ M	100 μ M 44.8 \pm 4.1
CBG-BDS	65.7 \pm 10.3	100 μ M 61.2 \pm 4.9
CBN	>50 μ M	50 μ M 26.9 \pm 3.8
CBDA	19.4 \pm 2.7	50 μ M 90.2 \pm 2.6
CBDA-BDS	21.8 \pm 1.3	50 μ M 87.4 \pm 2.4
CBGA	30.5 \pm 1.4	100 μ M 67.6 \pm 0.3
CBDV	16.6 \pm 4.1	100 μ M 83.3 \pm 1.8
CBDV-BDS	>50 μ M	50 μ M 16.2 \pm 6.3
CBGV	>50 μ M	50 μ M 18.9 \pm 6.9
CBGV-BDS	>50 μ M	50 μ M 47.1 \pm 8.5
THCA	27.3 \pm 1.6	50 μ M 86.2 \pm 6.9
THCA-BDS	21.8 \pm 2.8	50 μ M 97.6 \pm 1.4
THCV	>50 μ M	50 μ M 8.8 \pm 0.7
THCV-BDS	>50 μ M	50 μ M 0.5 \pm 0.1
THCVA	>50 μ M	100 μ M 71.6 \pm 5.1
THCVA-BDS	>50 μ M	100 μ M 52.1 \pm 10.3
CBDVA	35.0 \pm 5.6	50 μ M 61.0 \pm 0.7

Data are means \pm SEM of at least $n = 3$ separate experiments in which various concentrations (1, 10, 25, 50 or 100 μ M) of the pure compounds/BDS were incubated. Efficacy was calculated as % of DAGL α inhibition at the maximal concentration tested (which is shown in the first line of the cell). CBC, cannabichromene; CBD, cannabidiol; CBDA, cannabidiol acid; CBDV, propyl analogue of CBD or cannabidivarin; CBG, cannabigerol; CBGV, cannabigivarin; CBN, cannabinol; DAGL α , diacylglycerol lipase α ; THC, Δ^9 -tetrahydrocannabinol; THCA, THC acid; THCV, propyl analogue of THC or tetrahydrocannabivarin; THCVA, tetrahydrocannabivarin acid.

Table 6

Efficacy and potency of pure cannabinoids and of most of their corresponding 'botanical drug substance' (BDS) as inhibitors of COS cell MAGL

Compounds	IC ₅₀ (μ M)	Maximum concentration tested (% inhibition)
CBC	50.1 \pm 12.1	100 μ M 64.5 \pm 3.6
CBC-BDS	91.4 \pm 13.8	100 μ M 58.5 \pm 8.1
CBD	>100 μ M	100 μ M 0
CBD-BDS	>50 μ M	50 μ M 23.0 \pm 3.8
CBG	95.7 \pm 32.4	100 μ M 54.2 \pm 3.8
CBG-BDS	24.6 \pm 8.4	100 μ M 66.1 \pm 3.4
CBN	>50 μ M	50 μ M 31.5 \pm 3.3
CBDA	>50 μ M	50 μ M 18.5 \pm 2.1
CBDA-BDS	29.6 \pm 1.1	50 μ M 94.0 \pm 10.5
CBGA	>50 μ M	50 μ M 17.2 \pm 1.8
CBDV	>100 μ M	100 μ M 2.5 \pm 0.1
CBDV-BDS	>50 μ M	50 μ M 5.2 \pm 0.6
CBGV	>50 μ M	50 μ M 4.9 \pm 1.9
CBGV-BDS	>50 μ M	50 μ M 37.9 \pm 5.7
THCA	46.0 \pm 1.2	100 μ M 86.0 \pm 4.1
THCA-BDS	6.2 \pm 1.2	50 μ M 86.3 \pm 13.1
THCV	>50 μ M	50 μ M 18.2 \pm 5.4
THCV-BDS	>50 μ M	50 μ M 23.5 \pm 5.2
THCVA	>50 μ M	50 μ M 16.9 \pm 9.6
THCVA-BDS	>50 μ M	50 μ M 39.8 \pm 5.7

Data are means \pm SEM of at least $n = 3$ separate experiments in which various concentrations (1, 10, 25, 50 or 100 μ M) of the pure compounds/BDS were incubated. Efficacy was calculated as % of MAGL inhibition at the maximal concentration tested (which is shown in the first line of the cell). CBC, cannabichromene; CBD, cannabidiol; CBDA, cannabidiol acid; CBDV, propyl analogue of CBD or cannabidivarin; CBG, cannabigerol; CBGV, cannabigivarin; CBN, cannabinol; MAGL, monoacylglycerol lipase; THC, Δ^9 -tetrahydrocannabinol; THCA, THC acid; THCV, propyl analogue of THC or tetrahydrocannabivarin; THCVA, tetrahydrocannabivarin acid.

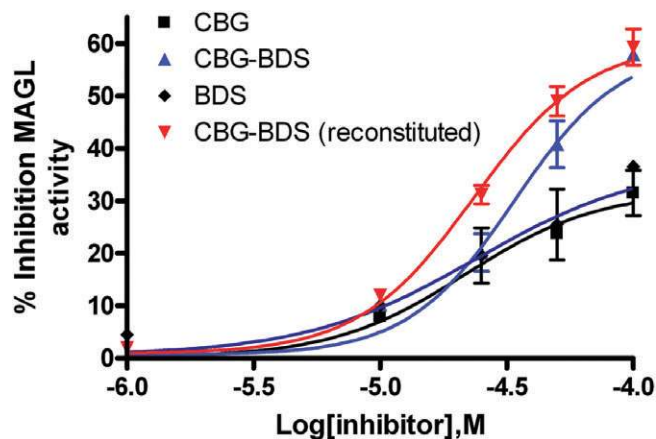


Figure 4

Inhibition of the effect of MAGL by cannabigerol (CBG), a CBG-botanical drug substance (CBG-BDS) as such or rendered free of CBG (denoted as BDS), and the reconstituted CBG-BDS (denoted as CBG-BDS reconstituted). Experiments were carried out in homogenates from COS cells and are means \pm SEM of at least $n = 3$ separate experiments. 'CBG-BDS reconstituted' was obtained by mixing the amount of CBG present in a given amount of CBG-BDS and the remaining amount of the 'CBG-free' BDS. The concentrations tested of 'CBG-BDS reconstituted' were calculated to contain equimolar concentrations of CBG as in pure CBG. BDS, botanical drug substance; CBG, cannabigerol; MAGL, monoacylglycerol lipase.

various TRPs seem to vary depending on the type of the channel, with CBC being the most potent at TRPA1 and the least potent at antagonizing TRPM8; CBN being relatively potent at TRPA1 and only weakly active at TRPV2; CBG and CBD the most potent at TRPM8 and TRPV1, and THC, THCV and CBD at TRPV2. With all TRP channels tested here, the cannabinoid acids were usually weakly active or inactive, whereas the propyl analogues were usually slightly less active than the corresponding pentyl cannabinoids. A notable exception to the former rule was the strong effect of THCA at TRPM8.

- Several cannabinoids also interfered at mid- μ M concentrations with enzymes catalyzing 2-AG biosynthesis via DAGL α , or with AEA inactivation via the putative membrane transporter or FAAH.
- The effects of the BDS at TRP channels reflected in many cases those of the corresponding pure cannabinoids. However, in some cases, these extracts were endowed with pharmacological actions of their own at the investigated targets, as in the case of the inhibitory effects observed on MAGL and NAAA, or with a capacity to inhibit AEA uptake, antagonize TRPM8 or activate TRPA1 that was stronger than that of the corresponding pure cannabinoids.

Our current findings extend the concept that some TRP channels may serve as ionotropic cannabinoid receptors, which, in the context of primary afferent nerve fibres, may contribute to inflammatory hypersensitivity or vasodilatation. These results were not completely unexpected, because we had found previously that some cannabinoids activate TRPV1 (Ligresti *et al.*, 2006) and TRPA1 (De Petrocellis *et al.*,

Table 7

Efficacy and potency of pure cannabinoids and of most of their corresponding 'botanical drug substance' (BDS) as inhibitors of rat brain FAAH

Compounds	IC ₅₀ (μ M)	Maximum concentration tested (% inhibition)
CBC	>100 μ M	100 μ M 46.5 \pm 1.3
CBC-BDS	53.2 \pm 11.3	100 μ M 68.6 \pm 4.1
CBD	15.2 \pm 3.2	50 μ M 87.2 \pm 4.1
CBD-BDS	30.8 \pm 6.2	100 μ M 89.4 \pm 2.5
CBG	>100 μ M	100 μ M 44.9 \pm 1.4
CBG-BDS	62.9 \pm 21.4	100 μ M 66.9 \pm 5.0
CBN	>50 μ M	50 μ M 29.1 \pm 3.3
CBDA	>50 μ M	50 μ M 42.2 \pm 4.0
CBDA-BDS	>50 μ M	50 μ M 43.9 \pm 6.0
CBGA	>50 μ M	50 μ M 46.2 \pm 4.9
CBDV	>100 μ M	100 μ M 36.2 \pm 3.1
CBDV-BDS	53.4 \pm 8.3	100 μ M 77.9 \pm 1.1
CBGV	>50 μ M	50 μ M 38.7 \pm 3.8
CBGV-BDS	>50 μ M	50 μ M 45.3 \pm 6.5
THCA	>50 μ M	50 μ M 38.8 \pm 3.1
THCA-BDS	>50 μ M	50 μ M 44.4 \pm 4.0
THCV	>100 μ M	100 μ M 26.8 \pm 3.4
THCV-BDS	>100 μ M	100 μ M 48.3 \pm 3.4
THCVA	>100 μ M	100 μ M 27.0 \pm 3.6
THCVA-BDS	>50 μ M	100 μ M 62.1 \pm 1.4

Data are means \pm SEM of at least $n = 3$ separate experiments in which various concentrations (1, 10, 25, 50 or 100 μ M) of the pure compounds/BDS were incubated. Efficacy was calculated as % of FAAH inhibition at the maximal concentration tested (which is shown in the first line of the cell). CBC, cannabichromene; CBD, cannabidiol; CBDA, cannabidiol acid; CBDV, propyl analogue of CBD or cannabidivarin; CBG, cannabigerol; CBN, cannabigvarin; CBN, cannabinol; FAAH, fatty acid amide hydrolase; THC, Δ^9 -tetrahydrocannabinol; THCA, THC acid; THCV, propyl analogue of THC or tetrahydrocannabivarin; THCVA, tetrahydrocannabivarin acid.

Table 8

Efficacy and potency of pure cannabinoids and of most of their corresponding 'botanical drug substance' (BDS) as inhibitors of human recombinant NAAA

Compounds	IC ₅₀ (μM)	Max concentration tested (% inhibition)
CBC	>100 μM	100 μM 40.6 ± 2.1
CBC-BDS	14.2 ± 6.2	100 μM 81.0 ± 5.1
CBD	>100 μM	100 μM 47.2 ± 2.6
CBD-BDS	>100 μM	100 μM 47.2 ± 3.4
CBG	>100 μM	100 μM 19.4 ± 2.1
CBG-BDS	18.3 ± 9.4	100 μM 68.5 ± 8.2
CBN	>50 μM	50 μM 31.4 ± 2.9
CBDA	23.0 ± 1.3	50 μM 62.5 ± 1.1
CBDA-BDS	>50 μM	50 μM 43.8 ± 8.4
CBGA	>50 μM	50 μM 33.2 ± 2.9
CBDV	72.3 ± 18.4	100 μM 54.5 ± 6.1
CBDV-BDS	>100 μM	100 μM 47.2 ± 8.2
CBGV	>50 μM	50 μM 38.4 ± 4.0
CBGV-BDS	24.4 ± 2.1	100 μM 75.0 ± 9.1
THCA	>50 μM	50 μM 21.1 ± 1.8
THCA-BDS	>50 μM	50 μM 25.5 ± 6.8
THCV	>100 μM	100 μM 18.2 ± 9.1
THCV-BDS	>100 μM	100 μM 21.4 ± 3.1
THCVA	>100 μM	100 μM 20.5 ± 3.5
THCVA-BDS	>100 μM	100 μM 44.7 ± 4.3

Data are means ± SEM of at least $n = 3$ separate experiments in which various concentrations (1, 10, 25, 50 or 100 μM) of the pure compounds/BDS were incubated. Efficacy was calculated as % of NAAA inhibition at the maximal concentration tested (which is shown in the first line of the cell). CBC, cannabichromene; CBD, cannabidiol; CBDA, cannabidiol acid; CBDV, propyl analogue of CBD or cannabidivarin; CBG, cannabigerol; CBGV, cannabigivarin; CBN, cannabinol; NAAA, N-acyl ethanolamine acid amide hydrolase; THC, Δ⁹-tetrahydrocannabinol; THCA, THC acid; THCV, propyl analogue of THC or tetrahydrocannabivarin; THCVA, tetrahydrocannabivarin acid.

Table 9

Efficacy and potency of pure cannabinoids and of most of their corresponding 'botanical drug substance' (BDS) as inhibitors of anandamide cellular uptake by RBL-2H3 cells

Compounds	IC ₅₀ (μM)	Max concentration tested (% inhibition)
CBC	12.3 ± 2.7	25 μM 83.2 ± 3.5
CBC-BDS	15.7 ± 4.2	25 μM 83.6 ± 7.8
CBD	25.3 ± 1.8	25 μM 49.9 ± 1.3
CBD-BDS	11.5 ± 3.2	25 μM 76.8 ± 5.3
CBG	11.3 ± 4.2	25 μM 85.4 ± 0.8
CBG-BDS	14.2 ± 2.5	25 μM 89.3 ± 2.7
CBN	~25	25 μM 50.0 ± 31.8
CBDA	>25	25 μM 36.3 ± 3.3
CBDA-BDS	5.8 ± 1.3	50 μM 70.3 ± 2.4
CBGA	>25	25 μM 43.6 ± 5.0
CBDV	21.3 ± 1.8	25 μM 52.7 ± 3.0
CBDV-BDS	>25	25 μM 16.4 ± 3.2
CBGV	>25	25 μM 31.2 ± 4.0
CBGV-BDS	6.9 ± 1.5	25 μM 78.7 ± 21.3
THCA	>25	25 μM 34.4 ± 2.8
THCA-BDS	9.7 ± 1.6	25 μM 90.0 ± 4.6
THCV	>25	25 μM 46.9 ± 2.0
THCV-BDS	>25	25 μM 49.3 ± 2.2
THCVA	>25	25 μM 47.2 ± 2.1
THCVA-BDS	12.5 ± 5.2	25 μM 71.0 ± 8.3

Data are means ± SEM of at least $n = 3$ separate experiments in which various concentrations (1, 5, 10, 25 μM) of the pure compounds/BDS were incubated. Efficacy was calculated as % of uptake inhibition at the maximal concentration tested (25 μM).

CBC, cannabichromene; CBD, cannabidiol; CBDA, cannabidiol acid; CBDV, propyl analogue of CBD or cannabidivarin; CBG, cannabigerol; CBGV, cannabigivarin; CBN, cannabinol; THC, Δ⁹-tetrahydrocannabinol; THCA, THC acid; THCV, propyl analogue of THC or tetrahydrocannabivarin; THCVA, tetrahydrocannabivarin acid.

2008) and antagonize TRPM8 (De Petrocellis *et al.*, 2008), and others have reported that THC and CBN activate TRPA1 (Jordt *et al.*, 2004), and that CBD activates TRPV2 (Qin *et al.*, 2008). However, here we investigated for the first time the activity at these four targets of all pure plant cannabinoids, and of all *Cannabis* BDS, available to date, thus allowing an initial structure–activity relationship study of the effects of these natural compounds at TRP channels, and a direct comparison with extracts that can be easily obtained from *Cannabis* strains specifically bred to produce one principal cannabinoid over the others. A limitation of our study is that we did not investigate the effects of the compounds on native cells expressing the TRP channels, such as, for example, sensory neurones from dorsal root or trigeminal ganglia, which are likely to possess a level of cellular structure and subcellular organization with respect to TRP channel localization different from that of HEK-293 cells. Furthermore, we did not assess, for example by using patch clamp techniques, whether or not the effects of the compounds are exerted directly on the channels. However, it must be remembered that all effects at TRP channels reported so far for plant and synthetic cannabinoids were shown to occur also in dorsal root ganglia neurones (Jordt *et al.*, 2004; De Petrocellis *et al.*, 2008; Qin *et al.*, 2008) and to be due to direct activation of the currents corresponding to these channels (Jordt *et al.*, 2004; Akopian *et al.*, 2008). Therefore, we have no reason to believe that the effects reported here are also not direct and do not occur in sensory neurones constitutively expressing the corresponding TRP channels. On the other hand, evidence has been obtained against cannabinoid interaction with TRP channels in cells with a level of expression of native TRP channels lower than in sensory neurones (Massi *et al.*, 2004; Drysdale *et al.*, 2006; Giudice *et al.*, 2007).

We have also shown here that the stimulating effects of cannabinoids at TRPA1, TRPV1 and TRPV2 result in the desensitization of these channels. In fact, as only those compounds that activated TRPV1 or TRPV2 or TRPA1 *per se* also antagonized the action of capsaicin or LPC or allyl isothiocyanates, respectively, it is unlikely that these effects were due to antagonism, as in the case of TRPM8, and not to channel desensitization, although clearly the former possibility cannot be completely ruled out. However, the potency for desensitization for a given cannabinoid was not always proportional to its potency as an agonist *per se*. Indeed, it is now well established that the ability of TRP agonists to also desensitize these channels depends not only on their potency but also on their lipophilicity and facility to penetrate and cross the plasma membrane (Morita *et al.*, 2006; Ursu *et al.*, 2010). Desensitization might have important consequences for the potential use of cannabinoids as therapeutic agents in those disorders in which these four channels have been shown to be implicated and to play a permissive role, such as chronic and inflammatory pain and cancer. It was reported previously that CBD evokes a concentration-dependent release of CGRP from cultured rat DRG neurones, which depended on extracellular calcium, was blocked by ruthenium red (a non-selective inhibitor of TRP channels) and was due to TRPA1 and TRPV2 activation (Qin *et al.*, 2008). Using selective knockdown of TRPV2 or TRPA1 mRNA levels, it was concluded that at least a significant component of the CBD-evoked CGRP release and antinociception was due to

activation and desensitization of TRPV2 respectively (Qin *et al.*, 2008). Importantly, synthetic cannabinoids that activate cannabinoid receptors were also shown to exert antinociceptive effects partly via TRPA1 desensitization (Akopian *et al.*, 2008).

Also, the inhibitory effects of CBDV, CBDA, CBGA, THCA and CBDVA on DAGL α might have important therapeutic implications. The inhibition of DAGL α with a selective synthetic inhibitor was recently found to inhibit the intake of palatable food compared with normal chow in mice (Bisogno *et al.*, 2009), and excessive 2-AG levels were associated with abdominal obesity and its metabolic consequences, such as dyslipidaemia and glucose intolerance (Di Marzo and Després, 2009). Therefore, if the above cannabinoids are also found to inhibit DAGL α *in vivo* and shown to lead to a reduction in the excessive 2-AG levels that accompany hyperphagia and abdominal obesity, one might propose the testing of, for example, CBDV, CBDA and CBDVA, in animal models of obesity and, eventually, in clinical trials in obese individuals.

As to the ability of THCA-BDS, CBDA-BDS and CBG-BDS to inhibit MAGL, this initially seemed to be due mostly to non-cannabinoid components of the extracts, as suggested by the fact that some plant natural products of terpenoid origin were recently found to potently inhibit this enzyme (King *et al.*, 2009), and by the observation that pure THCA, CBDA and CBG were significantly less potent, when not inactive, at inhibiting MAGL. However, when we tested a 'CBG-free' CBG-BDS, we also found it to be less active than CBG-BDS, thus suggesting that both cannabinoid and non-cannabinoid-components of *Cannabis* extracts might cooperate at inhibiting the enzyme. These findings indicate that these extracts might be used to inhibit chronic and inflammatory pain, because URB602, a synthetic compound with lower potency at MAGL than THCA-BDS, CBDA-BDS and CBG-BDS, was previously reported to act as an efficacious anti-hyperalgesic and anti-inflammatory compound in animal models (Hohmann *et al.*, 2005; Comelli *et al.*, 2007; Guindon *et al.*, 2007). A similar possibility might also exist for CBC-BDS, and CBGV-BDS as NAAA inhibitors, as synthetic compounds capable of inhibiting this enzyme, and subsequently of prolonging the lifespan of the anti-inflammatory endogenous endocannabinoid-like mediator, PEA, exert anti-inflammatory effects (Solorzano *et al.*, 2009; Petrosino *et al.*, 2010). In particular, CBG-BDS, due to its ability to inhibit both MAGL and NAAA, and antagonize TRPM8, should be tested in the future in animal models of chronic pain.

The use of several cannabinoids as analgesics is also suggested by their inhibitory effects on endocannabinoid inactivation mediated by either FAAH (which under certain conditions can also recognize 2-AG as a substrate) or AEA cellular reuptake (which occurs through an as yet uncharacterized mechanism that also recognizes 2-AG). Interestingly, several BDS and compounds were shown here to inhibit ACU even in the absence of any effect on FAAH, thus arguing in favour of the possibility that the re-uptake of endocannabinoids does not occur uniquely via FAAH-driven diffusion across the plasma membrane (Ligresti *et al.*, 2004).

In conclusion, our data support the current concept (Izzo *et al.*, 2009) that not only cannabinoids different from

THC (and CBD), but also cannabis extracts enriched in such compounds, might be used in the future as potential therapeutic agents, especially if the procedures for extraction of plant materials and the selection of the appropriate strains of *Cannabis* are standardized. Our findings also provide evidence that these natural products can interact with proteins of the ECS as well as with TRPV1, TRPV2, TRPA1 and TRPM8 channels. The present results also reinforce the suggestion that the latter channels, along with their role as sensors of thermic, inflammatory, mechanical and chemical stimuli, should be regarded as ionotropic cannabinoid receptors (Akopian *et al.*, 2009; De Petrocellis and Di Marzo, 2010). Finally, independently of their possible therapeutic implications, our present data suggest the use of some cannabinoids as pharmacological tools, especially for the study of those proteins, such as TRPV2 and TRPA1, for which very few selective agonists or antagonists have been reported. Future studies are needed to characterize those components of the various *Cannabis* extracts used here that inhibit the inactivation of EC and EC-like compounds, and the nature of their interactions with the effects of pure cannabinoids.

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Conflict of interests

VD is the recipient of a research grant from GW Pharma, Ltd. CGS is an employee of GW Pharma, Ltd.

References

- Akopian AN, Ruparel NB, Patwardhan A, Hargreaves KM (2008). Cannabinoids desensitize capsaicin and mustard oil responses in sensory neurons via TRPA1 activation. *J Neurosci* 28: 1064–1075.
- Akopian AN, Ruparel NB, Jeske NA, Patwardhan A, Hargreaves KM (2009). Role of ionotropic cannabinoid receptors in peripheral antinociception and antihyperalgesia. *Trends Pharmacol Sci* 30: 79–84.
- Bisogno T, Hanus L, De Petrocellis L, Tchilibon S, Ponde DE, Brandi I *et al.* (2001). Molecular targets for cannabidiol and its synthetic analogues: effect on vanilloid VR1 receptors and on the cellular uptake and enzymatic hydrolysis of anandamide. *Br J Pharmacol* 134: 845–852.
- Bisogno T, Howell F, Williams G, Minassi A, Cascio MG, Ligresti A *et al.* (2003). Cloning of the first sn1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signalling in the brain. *J Cell Biol* 163: 463–468.
- Bisogno T, Cascio MG, Saha B, Mahadevan A, Urbani P, Minassi A *et al.* (2006). Development of the first potent and specific inhibitors of endocannabinoid biosynthesis. *Biochim Biophys Acta* 1761: 205–212.
- Bisogno T, Burston JJ, Rai R, Allarà M, Saha B, Mahadevan A *et al.* (2009). Synthesis and pharmacological activity of a potent inhibitor of the biosynthesis of the endocannabinoid 2-arachidonoylglycerol. *ChemMedChem* 4: 946–950.
- Bolognini D, Costa B, Maione S, Comelli F, Marini P, Di Marzo V *et al.* (2010). The plant cannabinoid Δ^9 -tetrahydrocannabinol can decrease signs of inflammation and inflammatory pain in mice. *Br J Pharmacol* 160: 677–687.
- Campos AC, Guimarães FS (2008). Involvement of 5HT1A receptors in the anxiolytic-like effects of cannabidiol injected into the dorsolateral periaqueductal gray of rats. *Psychopharmacology* 199: 223–230.
- Capasso R, Borrelli F, Aviello G, Romano B, Scalisi C, Capasso F *et al.* (2008). Cannabidiol, extracted from *Cannabis sativa*, selectively inhibits inflammatory hypermotility in mice. *Br J Pharmacol* 154: 1001–1008.
- Carrier EJ, Auchampach JA, Hillard CJ (2006). Inhibition of an equilibrative nucleoside transporter by cannabidiol: a mechanism of cannabinoid immunosuppression. *Proc Natl Acad Sci USA* 103: 7895–7900.
- Cascio MG, Gauson LA, Stevenson LA, Ross RA, Pertwee RG (2010). Evidence that the plant cannabinoid cannabigerol is a highly potent α 2-adrenoceptor agonist and moderately potent 5HT1A receptor antagonist. *Br J Pharmacol* 159: 129–141.
- Comelli F, Giagnoni G, Bettoni I, Colleoni M, Costa B (2007). The inhibition of monoacylglycerol lipase by URB602 showed an anti-inflammatory and anti-nociceptive effect in a murine model of acute inflammation. *Br J Pharmacol* 152: 787–794.
- Costa B, Colleoni M, Conti S, Parolaro D, Franke C, Trovato AE *et al.* (2004). Oral anti-inflammatory activity of cannabidiol, a non-psychoactive constituent of cannabis, in acute carrageenan-induced inflammation in the rat paw. *Naunyn Schmiedebergs Arch Pharmacol* 369: 294–299.
- Costa B, Trovato AE, Comelli F, Giagnoni G, Colleoni M (2007). The non-psychoactive cannabis constituent cannabidiol is an orally effective therapeutic agent in rat chronic inflammatory and neuropathic pain. *Eur J Pharmacol* 556: 75–83.
- Cravatt BF, Giang DK, Mayfield SP, Boger DL, Lerner RA, Gilula NB (1996). Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. *Nature* 384: 83–87.
- De Petrocellis L, Di Marzo V (2010). Non-CB1, non-CB2 receptors for endocannabinoids, plant cannabinoids, and synthetic cannabimimetics: focus on G-protein-coupled receptors and transient receptor potential channels. *J Neuroimmune Pharmacol* 5: 103–121.
- De Petrocellis L, Starowicz K, Schiano-Moriello A, Vivese M, Orlando P, Di Marzo V (2007). Regulation of transient receptor potential channels of melastatin type 8 (TRPM8): effect of cAMP, cannabinoid CB(1) receptors and endovanilloids. *Exp Cell Res* 313: 1911–1920.
- De Petrocellis L, Vellani V, Schiano-Moriello A, Marini P, Magherini PC, Orlando P *et al.* (2008). Plant-derived cannabinoids modulate the activity of transient receptor potential channels of ankyrin type-1 and melastatin type-8. *J Pharmacol Exp Ther* 325: 1007–1015.
- Delong GT, Wolf CE, Poklis A, Lichtman AH (2010). Pharmacological evaluation of the natural constituent of *Cannabis sativa*, cannabichromene and its modulation by Δ^9 -tetrahydrocannabinol. *Drug Alcohol Depend* 112: 126–133.
- Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, Gibson D *et al.* (1992). Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* 258: 1946–1949.

- Di Marzo V, Després JP (2009). CB1 antagonists for obesity – what lessons have we learned from rimonabant? *Nat Rev Endocrinol* 5: 633–638.
- Di Marzo V, Fontana A, Cadas H, Schinelli S, Cimino G, Schwartz JC *et al.* (1994). Formation and inactivation of endogenous cannabinoid anandamide in central neurons. *Nature* 372: 686–691.
- Di Marzo V, De Petrocellis L, Bisogno T (2005). The biosynthesis, fate and pharmacological properties of endocannabinoids. In: Pertwee RG (ed.). *Cannabinoids. Handbook of Experimental Pharmacology*, Vol. 168. Springer-Verlag: Heidelberg, pp. 147–185.
- Dinh TP, Carpenter D, Leslie FM, Freund TF, Katona I, Sensi SL *et al.* (2002). Brain monoglyceride lipase participating in endocannabinoid inactivation. *Proc Natl Acad Sci USA* 99: 10819–10824.
- Drysdale AJ, Ryan D, Pertwee RG, Platt B (2006). Cannabidiol-induced intracellular Ca^{2+} elevations in hippocampal cells. *Neuropharmacology* 50: 621–631.
- ElSohly MA, Slade D (2005). Chemical constituents of marijuana: the complex mixture of natural cannabinoids. *Life Sci* 78: 539–548.
- Gaoni Y, Mechoulam R (1964). Isolation, structure, and partial synthesis of an active constituent of hashish. *J Am Chem Soc* 86: 1646–1647.
- García-Arencibia M, González S, de Lago E, Ramos JA, Mechoulam R, Fernández-Ruiz J (2007). Evaluation of the neuroprotective effect of cannabinoids in a rat model of Parkinson's disease: importance of antioxidant and cannabinoid receptor-independent properties. *Brain Res* 1134: 162–170.
- Giudice ED, Rinaldi L, Passarotto M, Facchinetti F, D'Arrigo A, Guiotto A *et al.* (2007). Cannabidiol, unlike synthetic cannabinoids, triggers activation of RBL-2H3 mast cells. *J Leukoc Biol* 81: 1512–1522.
- Gong J-P, Onaivi ES, Ishiguro H, Liu Q-R, Tagliaferro PA, Brusco A *et al.* (2006). Cannabinoid CB2 receptors: immunohistochemical localization in rat brain. *Brain Res* 1071: 10–23.
- Guindon J, Desroches J, Beaulieu P (2007). The antinociceptive effects of intraplantar injections of 2-arachidonoyl glycerol are mediated by cannabinoid CB2 receptors. *Br J Pharmacol* 150: 693–701.
- Hatoum NS, Davis WM, Elsohly MA, Turner CE (1981). Cannabichromene and Δ^9 -tetrahydrocannabinol: interactions relative to lethality, hypothermia and hexobarbital hypnosis. *Gen Pharmacol* 12: 357–362.
- Hayakawa K, Mishima K, Hazekawa M, Sano K, Irie K, Orito K *et al.* (2008). Cannabidiol potentiates pharmacological effects of Δ^9 -tetrahydrocannabinol via CB(1) receptor-dependent mechanism. *Brain Res* 1188: 157–164.
- Hohmann AG, Suplita RL, Bolton NM, Neely MH, Fegley D, Mangieri R *et al.* (2005). An endocannabinoid mechanism for stress-induced analgesia. *Nature* 435: 1108–1112.
- Iuvone T, Esposito G, De Filippis D, Scuderi C, Steardo L (2009). Cannabidiol: a promising drug for neurodegenerative disorders? *CNS Neurosci Ther* 15: 65–75.
- Izzo AA, Borrelli F, Capasso R, Di Marzo V, Mechoulam R (2009). Non-psychoactive plant cannabinoids: new therapeutic opportunities from an ancient herb. *Trends Pharmacol Sci* 30: 515–527.
- Jordt SE, Bautista DM, Chuang HH, McKemy DD, Zygmunt PM, Högestätt ED *et al.* (2004). Mustard oils and cannabinoids excite sensory nerve fibres through the TRP channel ANKTM1. *Nature* 427: 260–265.
- King AR, Dotsey EY, Lodola A, Jung KM, Ghomian A, Qiu Y *et al.* (2009). Discovery of potent and reversible monoacylglycerol lipase inhibitors. *Chem Biol* 16: 1045–1052.
- Levine JD, Alessandri-Haber N (2007). TRP channels: targets for the relief of pain. *Biochim Biophys Acta* 1772: 989–1003.
- Ligresti A, Morera E, Van Der Stelt M, Monory K, Lutz B, Ortas G *et al.* (2004). Further evidence for the existence of a specific process for the membrane transport of anandamide. *Biochem J* 380: 265–272.
- Ligresti A, Schiano-Moriello A, Starowicz K, Matias I, Pisanti S, De Petrocellis L *et al.* (2006). Antitumor activity of plant cannabinoids with emphasis on the effect of cannabidiol on human breast carcinoma. *J Pharmacol Exp Ther* 318: 1375–1387.
- Ligresti A, De Petrocellis L, Hernán Pérez de la Ossa D, Aberturas R, Cristino L, Schiano-Moriello A *et al.* (2010). Exploiting nanotechnologies and TRPV1 channels to investigate the putative anandamide membrane transporter. *PLoS One* 5: e10239.
- McNamara CR, Mandel-Brehm J, Bautista DM, Siemens J, Deranian KL, Zhao M *et al.* (2007). TRPA1 mediates formalin-induced pain. *Proc Natl Acad Sci USA* 104: 13525–13530.
- Magen I, Avraham Y, Ackerman Z, Vorobiev L, Mechoulam R, Berry EM (2010). Cannabidiol ameliorates cognitive and motor impairments in bile-duct ligated mice via 5-HT1A receptor activation. *Br J Pharmacol* 159: 950–957.
- Marcu JP, Christian RT, Lau D, Zielinski AJ, Horowitz MP, Lee J *et al.* (2010). Cannabidiol enhances the inhibitory effects of Δ^9 -tetrahydrocannabinol on human glioblastoma cell proliferation and survival. *Mol Cancer Ther* 9: 180–189.
- Massi P, Vaccani A, Ceruti S, Colombo A, Abbraccio MP, Parolaro D (2004). Antitumor effects of cannabidiol, a nonpsychoactive cannabinoid, on human glioma cell lines. *J Pharmacol Exp Ther* 308: 838–845.
- Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI (1990). Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature* 346: 561–564.
- Mechoulam R (2000). Looking back at Cannabis research. *Curr Pharm Des* 6: 1313–1322.
- Mechoulam R, Shvo Y (1963). Hashish – I: the structure of cannabidiol. *Tetrahedron* 19: 2073–2078.
- Mechoulam R, Ben-Shabat S, Hanus L, Ligumsky M, Kaminski NE, Schatz AR *et al.* (1995). Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem Pharmacol* 50: 83–90.
- Mehmedic Z, Chandra S, Slade D, Denham H, Foster S, Patel AS *et al.* (2010). Potency trends of $\Delta(9)$ -THC and other cannabinoids in confiscated cannabis preparations from 1993 to 2008. *J Forensic Sci* 55: 1209–1217.
- Monet M, Gkika D, Lehen'kyi V, Poutier A, Vanden Abeele F, Bidaux G *et al.* (2009). Lysophospholipids stimulate prostate cancer cell migration via TRPV2 channel activation. *Biochim Biophys Acta* 1793: 528–539.
- Morita A, Iwasaki Y, Kobata K, Iida T, Higashi T, Oda K *et al.* (2006). Lipophilicity of capsaicinoids and capsinoids influences the multiple activation process of rat TRPV1. *Life Sci* 79: 2303–2310.

- Munro S, Thomas KL, Abu SM (1993). Molecular characterization of a peripheral receptor for cannabinoids. *Nature* 365: 61–65.
- Neeper MP, Liu Y, Hutchinson TL, Wang Y, Flores CM, Qin N (2007). Activation properties of heterologously expressed mammalian TRPV2: evidence for species dependence. *J Biol Chem* 282: 15894–15902.
- Pan H, Mukhopadhyay P, Rajesh M, Patel V, Mukhopadhyay B, Gao B *et al.* (2009). Cannabidiol attenuates cisplatin-induced nephrotoxicity by decreasing oxidative/nitrosative stress, inflammation, and cell death. *J Pharmacol Exp Ther* 328: 708–714.
- Pertwee RG (2004). The pharmacology and therapeutic potential of cannabidiol. In: Di Marzo V (ed.). *Cannabinoids*. Kluwer Academic/Plenum Publishers: London, pp. 32–83.
- Pertwee RG (2008). The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: Δ^9 -tetrahydrocannabinol, cannabidiol and Δ^9 -tetrahydrocannabivarin. *Br J Pharmacol* 153: 199–215.
- Petrosino S, Iuvone T, Di Marzo V (2010). N-palmitoyl-ethanolamine: biochemistry and new therapeutic opportunities. *Biochimie* 92: 724–727.
- Petrus M, Peier AM, Bandell M, Hwang SW, Huynh T, Olney N *et al.* (2007). A role of TRPA1 in mechanical hyperalgesia is revealed by pharmacological inhibition. *Mol Pain* 3: 40.
- Qin N, Nepper MP, Liu Y, Hutchinson TL, Lubin ML, Flores CM (2008). TRPV2 is activated by cannabidiol and mediates CGRP release in cultured rat dorsal root ganglion neurons. *J Neurosci* 28: 6231–6238.
- Resstel LB, Tavares RF, Lisboa SF, Joca SR, Corrêa FM, Guimarães FS (2009). 5-HT1A receptors are involved in the cannabidiol-induced attenuation of behavioural and cardiovascular responses to acute restraint stress in rats. *Br J Pharmacol* 156: 181–188.
- Russo E, Guy GW (2006). A tale of two cannabinoids: the therapeutic rationale for combining tetrahydrocannabinol and cannabidiol. *Med Hypotheses* 66: 234–246.
- Russo EB, Burnett A, Hall B, Parker KK (2005). Agonistic properties of cannabidiol at 5-HT1a receptors. *Neurochem Res* 30: 1037–1043.
- Ryan D, Drysdale AJ, Lafourcade C, Pertwee RG, Platt B (2009). Cannabidiol targets mitochondria to regulate intracellular Ca^{2+} levels. *J Neurosci* 29: 2053–2063.
- Saturnino C, Petrosino S, Ligresti A, Palladino C, De Martino G, Bisogno T *et al.* (2010). Synthesis and biological evaluation of new potential inhibitors of N-acylethanolamine hydrolyzing acid amidase. *Bioorg Med Chem Lett* 20: 1210–1213.
- Sofia RD, Nalepa SD, Vassar HB, Knobloch LC (1974). Comparative anti-phlogistic activity of Δ^9 -tetrahydrocannabinol, hydrocortisone and aspirin in various rat paw edema models. *Life Sci* 15: 251–260.
- Solorzano C, Zhu C, Battista N, Astarita G, Lodola A, Rivara S *et al.* (2009). Selective N-acylethanolamine-hydrolyzing acid amidase inhibition reveals a key role for endogenous palmitoylethanolamide in inflammation. *Proc Natl Acad Sci USA* 106: 20966–20971.
- Sugiura T, Kondo S, Sukagawa A, Nakane S, Shinoda A, Itoh K *et al.* (1995). 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. *Biochem Biophys Res Comm* 215: 89–97.
- Thomas A, Stevenson LA, Wease KN, Price MR, Baillie G, Ross RA *et al.* (2005). Evidence that the plant cannabinoid Δ^9 -tetrahydrocannabivarin is a cannabinoid CB1 and CB2 receptor antagonist. *Br J Pharmacol* 146: 917–926.
- Thomas A, Baillie GL, Phillips AM, Razdan RK, Ross RA, Pertwee RG (2007). Cannabidiol displays unexpectedly high potency as an antagonist of CB1 and CB2 receptor agonists in vitro. *Br J Pharmacol* 150: 613–623.
- Turner CE, Elsohly MA (1981). Biological activity of cannabichromene, its homologs and isomers. *J Clin Pharmacol* 21: 283S–291S.
- Ueda N, Yamanaka K, Yamamoto S (2001). Purification and characterization of an acid amidase selective for N-palmitoylethanolamine, a putative endogenous anti-inflammatory substance. *J Biol Chem* 276: 35552–35557.
- Ueda N, Tsuboi K, Uyama T (2010). N-acylethanolamine metabolism with special reference to N-acylethanolamine-hydrolyzing acid amidase (NAAA). *Prog Lipid Res* 49: 299–315.
- Ursu D, Knopp K, Beattie RE, Liu B, Sher E (2010). Pungency of TRPV1 agonists is directly correlated with kinetics of receptor activation and lipophilicity. *Eur J Pharmacol* 641: 114–122.
- Van Sickle MD, Duncan M, Kingsley PJ, Mouihate A, Urbani P, Mackie K *et al.* (2005). Identification and functional characterization of brainstem cannabinoid CB2 receptors. *Science* 310: 329–332.
- Varvel SA, Wiley JL, Yang R, Bridgen DT, Long K, Lichtman AH *et al.* (2006). Interactions between THC and cannabidiol in mouse models of cannabinoid activity. *Psychopharmacology* 186: 226–234.
- Watanabe K, Ogi H, Nakamura S, Kayano Y, Matsunaga T, Yoshimura H *et al.* (1998). Distribution and characterization of anandamide amidohydrolase in mouse brain and liver. *Life Sci* 62: 1223–1229.
- Yang KH, Galadari S, Isaev D, Petroianu G, Shippenberg TS, Oz M (2010). The nonpsychoactive cannabinoid cannabidiol inhibits 5-hydroxytryptamine3A receptor-mediated currents in *Xenopus laevis* oocytes. *J Pharmacol Exp Ther* 333: 547–554.