Voltage-gated sodium (NaV) channel blockade by plant cannabinoids does not confer anticonvulsant effects per se

Article *in* Neuroscience Letters · March 2014

DOI: 10.1016/j.neulet.2014.03.013 · Source: PubMed

CITATIONS
34

READS
464

7 authors, including:

Imogen Smith
University of Reading
8 PUBLICATIONS **368** CITATIONS
SEE PROFILE

Charlotte L Hill
University College Dublin
5 PUBLICATIONS **613** CITATIONS
SEE PROFILE

Claire M Williams
University of Reading
104 PUBLICATIONS **4,881** CITATIONS
SEE PROFILE

Gary John Stephens
University of Reading
86 PUBLICATIONS **3,029** CITATIONS
SEE PROFILE

Neuroscience Letters 566 (2014) 269–274

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Voltage-gated sodium (Na_V) channel blockade by plant cannabinoids does not confer anticonvulsant effects *per se*

Andrew J. Hill[a,b], Nicholas A. Jones[a,b,1], Imogen Smith[a], Charlotte L. Hill[a,b], Claire M. Williams[b], Gary J. Stephens[a,2], Benjamin J. Whalley[a,*,2]

[a] *Reading School of Pharmacy, University of Reading, Whiteknights, Reading RG6 6AJ, UK*
[b] *School of Psychology and Clinical Language Sciences, University of Reading, Whiteknights, Reading RG6 6AJ, UK*

HIGHLIGHTS

- Cannabidiol (CBD) and cannabigerol (CBG) block Na^+ channels in neurons *in vitro*.
- CBG does not affect pentyleneterazole-induced (PTZ) generalised seizures.
- *In vitro* NaV channel block by CBG does not correlate with anticonvulsant effects.
- NaV channel blocking cannabinoids cannot be presumed to be anticonvulsant.

ARTICLE INFO

Article history:
Received 3 December 2013
Received in revised form 27 January 2014
Accepted 1 March 2014

Keywords:
Epilepsy
Cannabinoid
Seizure
Sodium channels
Anticonvulsant
Dravet syndrome

ABSTRACT

Cannabidiol (CBD) is a non-psychoactive, well-tolerated, anticonvulsant plant cannabinoid, although its mechanism(s) of seizure suppression remains unknown. Here, we investigate the effect of CBD and the structurally similar cannabinoid, cannabigerol (CBG), on voltage-gated Na^+ (Na_V) channels, a common anti-epileptic drug target. CBG's anticonvulsant potential was also assessed *in vivo*. CBD effects on Na_V channels were investigated using patch-clamp recordings from rat CA1 hippocampal neurons in brain slices, human SH-SY5Y (neuroblastoma) cells and mouse cortical neurons in culture. CBG effects were also assessed in SH-SY5Y cells and mouse cortical neurons. CBD and CBG effects on veratridine-stimulated human recombinant $Na_V1.1$, 1.2 or 1.5 channels were assessed using a membrane potential-sensitive fluorescent dye high-throughput assay. The effect of CBG on pentyleneterazole-induced (PTZ) seizures was assessed in rat. CBD (10 μM) blocked Na_V currents in SH-SY5Y cells, mouse cortical neurons and recombinant cell lines, and affected spike parameters in rat CA1 neurons; CBD also significantly decreased membrane resistance. CBG blocked Na_V to a similar degree to CBD in both SH-SY5Y and mouse recordings, but had no effect (50–200 mg/kg) on PTZ-induced seizures in rat. CBD and CBG are Na_V channel blockers at micromolar concentrations in human and murine neurons and recombinant cells. In contrast to previous reports investigating CBD, CBG had no effect upon PTZ-induced seizures in rat, indicating that Na_V blockade *per se* does not correlate with anticonvulsant effects.

1. Introduction

CBD, a plant cannabinoid ('phytocannabinoid'; pCB) [1] that exerts diverse pharmacological effects [2,3] is anticonvulsant. CBD suppresses audiogenic, maximal-, 6 Hz- and 60 Hz-electroshock, cobalt-, picrotoxin-, 3-mercaptopropionic acid-, isonicotinic acid-, bicuculline-, hydrazine-, strychnine-, pentylenetetrazole (PTZ)-, pilocarpine- and penicillin-induced seizures in murine species [4–6]. CBD can also treat human epilepsies [7] and is now in use in intractable childhood epilepsy [8]. However, CBD's mechanism(s) of seizure suppression are unknown. Unlike the principal pCB, Δ^9-tetrahydrocannabinol, CBD has little significant activity

Abbreviations: AED, antiepileptic drug; CBD, cannabidiol; CBG, cannabigerol; CHL, Chinese hamster lung; CHO, Chinese hamster ovary; IFF, instantaneous spike firing frequency; Na_V, voltage-gated Na^+ channels; PTZ, pentylenetetrazole.

* Corresponding author. +44 118 378 4745.
E-mail address: b.j.whalley@reading.ac.uk (B.J. Whalley).
[1] Present address: GW Pharmaceuticals plc, Porton Down Science Park, Salisbury, Wiltshire SP4 0JQ, UK.
[2] Both authors contributed equally to this paper.

http://dx.doi.org/10.1016/j.neulet.2014.03.013
0304-3940/

at CB1 cannabinoid receptors [2,7], but does act at several ion channels. CBD blocks $Ca_V3.1$ and 3.2 voltage-gated Ca^{2+} channels and native neuronal T-type Ca^{2+} currents [9]. Moreover, CBD activates vanilloid transient receptor potential channels TRPV1, 2 and 3 and the ankyrin subfamily member TRPA1 (prolonged exposure causes desensitisation) [10–12], in addition to antagonising TRPM8 (melastatin-type [11]).

Existing antiepileptic drugs (AEDs) have a variety of targets [13] including receptors, synaptic machinery and ion channels such as Na_V. Given CBD's propensity to affect ion channels, we examined whether CBD affects Na_V channels at concentrations approximating brain levels after administration of effective anticonvulsant doses [6,14], and to what extent this could underlie CBD's anticonvulsant effect. We used patch-clamp electrophysiology across three preparations: rat acute hippocampal brain slices, a human neuroblastoma cell line (SH-SY5Y [15]) and primary cultures of embryonic mouse cortical neurons. We also used a voltage-sensitive dye fluorescence assay to study CBD effects at human $Na_V1.1$, 1.2 and 1.5 subtypes. Importantly, we also investigated a second, structurally similar, pCB, cannabigerol (CBG). CBG's pharmacological profile is less well-defined than CBD's, but is reported to be an α_2-adrenoceptor agonist and $5HT_{1A}$ receptor antagonist [16]. CBG's anticonvulsant potential has not previously been determined. Here we report that CBG is not anticonvulsant in generalised seizures in rat. This finding allowed us to assess pCB actions at Na_V channels and compare the effects of anticonvulsant CBD with those of CBG, which does not suppress seizure activity. We conclude that Na_V blocking cannabinoids cannot be presumed to be anti-convulsant.

2. Methods

2.1. Chemicals, reagents and animals

CBD and CBG were from GW Pharmaceuticals (Salisbury, UK). Unless stated, other chemicals and reagents were from Sigma (Poole, UK). Animals (Wistar–Kyoto rats and timed-mated female mice) were from Harlan (Bicester, UK). Animal experiments were performed in accordance with the Animals (Scientific Procedures) Act, 1986 and ARRIVE guidelines [17]. 72 rats were used for *in vivo* seizure experiments, 13 to produce brain slices and 9 mice to produce cultures.

2.2. Brain slice preparation

Transverse hippocampal slices (~300 μm thick) for patch-clamp recordings were prepared from female and male adult Wistar–Kyoto rats ($p > 23$; Harlan, UK) using a Vibroslice 725M (Campden Instruments Ltd., Loughborough, UK) as previously described [6].

2.3. Cell culture

All cells were maintained at 37 °C/5% CO_2 in a humidified incubator.

2.3.1. Mouse cortical neurons

Timed-mated NIHS female mice (Harlan) were sacrificed, E13-15 embryos removed and decapitated into phosphate-buffered solution containing 33 mM glucose. Embryo cortices were isolated, mechanically dissociated using a fire-polished glass Pasteur pipette, and the resultant suspension allowed to settle before the supernatant (containing cells) was removed and spun for 5 min at 200 x *g*. The resultant pellet was resuspended ($1.5–2.0 \times 10^5$ ml^{-1} viable cells) in culture medium (Eagle's minimal essential medium (EMEM)) supplemented with 5% heat-inactivated horse serum, 0.5 mM L-glutamine, 15 mM glucose and 10 μg/ml gentamicin sulphate (all Lonza, Slough, UK). Cells were seeded (1 ml/well) in multi-well plates containing poly-D-lysine-coated cover slips. Half the media was replaced every 3–4 days until cells were used for recording (4–11 days after dissociation).

2.3.2. Human neuroblastoma cells

SH-SY5Y cells (passage 12–23; passaged at 70% confluency) were maintained in DMEM/F12 medium (Dulbecco's Modified Eagle Medium) supplemented with 10 μg/ml gentamicin sulphate, 1% non-essential amino acids and 10% foetal bovine serum (all Lonza). 3–7 days prior to recording, cells were reseeded in media containing 1% serum and 10 μM all-*trans* retinoic acid. For recordings, cells were treated with enzyme-free cell dissociation buffer (Lonza), re-plated onto glass coverslips, left to adhere and placed in the recording chamber.

2.3.3. hNa_V cell lines

$hNa_V1.1$ and $hNa_V1.2$ were stably expressed in division-arrested Chinese hamster ovary (CHO) cells (Chantest, Ohio, USA) and plated onto 384-well assay plates (Corning, NY, USA) in Ham's F12 medium (Invitrogen Ltd) supplemented with 10% FBS, 1% geneticin and 1% penicillin–streptomycin (all Lonza); $hNa_V1.5$ stably expressed in Chinese hamster lung (CHL) cells (Molecular Devices (UK) Ltd, Wokingham, UK) was cultured using Dulbecco's modified eagle's medium (DMEM) supplemented with 10% foetal bovine serum (Biosera, East Sussex, UK), 1% genetecin (Invitrogen Ltd, Paisley, UK) and 1% PEN-streptomycin (Lonza).

2.4. Patch-clamp recordings

Due to the range of preparations and assays, several amplifiers were used for patch-clamp recordings: EPC10 amplifier (HEKA Electronik, Lambrecht, Germany; CBD + rat hippocampal brain slices); EPC9 amplifier (HEKA; SH-SY5Y and CBD + cortical neurons); EPC7 amplifier (HEKA; CBG + cortical neurons). Data were acquired using PatchMaster or WinWCP (John Dempster, Strathclyde University, UK). Electrodes were pulled from borosilicate glass (GC150-F10; Harvard Apparatus, Cambridge, UK) using a P87 Flaming Brown Micropipette Puller (Sutter Instruments Co., California, USA) and had resistances of 4–8 MΩ, dependent on intracellular solution and recording type. The pipette solution for brain slice recordings comprised (mM): K-gluconate (140); K_2ATP (2); NaGTP (0.1); $MgCl_2$ (1); HEPES (10) adjusted to pH 7.25 with KOH. For SH-SY5Y cell and cortical neuron voltage-clamp recordings (mM): CsCl (110); NaCl (10); HEPES (5); $MgCl_2$ (1); $CaCl_2$ (0.1); EGTA (10) adjusted to pH 7.3 with CsOH. Extracellular solution for brain slice recordings was continuously bubbled with 95% O_2/5% CO_2 and comprised (mM): NaCl (124); KCl (3); KH_2PO_4 (1.25); $NaHCO_3$ (36); MgSO4 (1) D-glucose (10) $CaCl_2$ (2). SH-SY5Y and cortical neurons extracellular solution (mM): NaCl (140); KCl (5); $CaCl_2$ (2); HEPES (10); glucose (10); $MgCl_2$ (2), adjusted to pH 7.3 with Sigma 7–9; extracellular solution for cortical neurons, but not SH-SY5Y cells, also contained 2 μM glycine. In voltage-clamp, holding potential was −80 mV. Drug effects were assessed after ≥20 min by voltage-step to −10 mV (peak Na_V current) for 20 ms. CA1 hippocampal neurons in rat brain slices were recorded in current-clamp configuration. Cells were held at −82 mV and passive and active membrane properties assessed using hyperpolarizing and depolarizing current pulses (−350 to 350 pA; 150 ms) before and after 30 min superfusion with CBD. Instantaneous firing frequencies (IFF) are the reciprocal of peak-to-peak inter-event intervals.

CBD and CBG were dissolved in DMSO or ethanol and added to the extracellular solution (maximum DMSO/ethanol concentration 0.1%). CBD was investigated at a known anticonvulsant concentration: CBD is anticonvulsant in rat at 100 mg/kg (i.p.; 1 h before

challenge) against PTZ-induced seizures [5,6]. 120 mg/kg CBD (i.p.) yields a brain concentration of 9.8 μM after 1 h, rising to 21.6 μM (C_{max}) an hour later [6]. Therefore, 1 or 10 μM CBD concentrations were used in electrophysiological experiments. CBG exhibits comparable pharmacokinetics [14].

2.5. Fluorescent imaging plate reader (FLIPR) assay

A Flexstation 3 (Molecular Devices) microplate reader measured fluorescence changes in recombinant cell lines expressing hNa_V subtypes (1.1, 1.2 or 1.5) incubated with a membrane voltage-sensitive dye (FLIPR Membrane Potential (FMP) dye, Molecular Devices). After plating at 12,500 cells/well, cells were exposed to the Na_V channel opening drug veratridine (0.391–200 μM); a veratridine concentration that elicited an 80% maximal fluorescence response (EC_{80}) was determined for each cell line and used thereafter. CBD and CBG were dissolved in DMSO and added to extracellular solution (maximum DMSO concentration 0.1%). When assessing CBD (0.001–200 μM), CBG (0.001–200 μM), and lidocaine (3–5000 μM; positive control) effects, FMP dye was excited at 530 nm and sampled at 565 nm every 1.5 s. Baseline fluorescence was measured for 28 s before drug application, then for 100 s after application of CBD, CBG or lidocaine ($n = 3$/drug/concentration) and finally for 100 s after addition of veratridine. Fluorescence changes in the presence of veratridine alone or veratridine + test compounds were expressed as relative fluorescence units. The effects of test compounds were expressed as a percentage inhibition of the veratridine (EC_{80}) control response and concentration-response curves fitted using nonlinear regression in Prism 4 (Graphpad Software, San Diego, California).

2.6. PTZ seizure model

Using male Wistar–Kyoto rats (Harlan; P23-28), PTZ seizures were induced, recorded and analysed as previously described [18]. CBG (50–200 mg/kg) and CBG vehicle (2:1:17 ethanol:Cremophor:0.9% (w/v) NaCl) were administered i.p. 1 h prior to i.p. 85 mg/kg PTZ.

3. Results

3.1. CBD effects on post-synaptic stimulation in rat CA1 hippocampal neurons

10 μM CBD affected postsynaptic membrane resistance and spike firing of CA1 pyramidal neurons stimulated by current injection (Fig. 1A). CBD significantly decreased number of spikes evoked by a 150 ms/350 pA depolarizing current (5.3 ± 0.4 to 3.1 ± 0.4; $p \leq 0.001$; $n = 8$ throughout), with a concomitant decrease in spike frequency (35 ± 2.4 Hz to 21 ± 2.3 Hz; $p \leq 0.001$). CBD (10 μM) also significantly increased first (5.96 ± 0.53 ms to 8.33 ± 1.01 ms; $p \leq 0.05$) and second (10.61 ± 0.55 ms to 24.13 ± 2.19 ms; $p \leq 0.001$) spike duration. IFF was not decreased between spikes 1–2 ($p \leq 0.1$), IFF was significantly decreased between spikes 2–3 (40.6 ± 4.3 Hz to 22.5 ± 3.2 Hz; $p \leq 0.01$). CBD significantly decreased steady-state membrane resistance (105.0 ± 16.8 MΩ to 72.4 ± 6.6 MΩ; $p \leq 0.05$), and increased the minimum stimulus required to evoke spiking (modal threshold from 100 pA to 200 pA; $p \leq 0.05$). CBD effects were retained in the presence of the CB1 antagonist SR141716A (2 μM; Fig. 1B; $n = 5$), with the exception of the decreased membrane resistance. However, in SR141716A alone, steady-state membrane resistance increased (126.3 ± 35.0 MΩ to 136.2 ± 27.0 MΩ; $p > 0.05$), a change opposite in direction from that caused by CBD alone, which may account for the apparent loss of this CBD effect. SR141716A exerted no significant effect on any of the other parameters measured (data not shown).

3.2. CBD effects on Na^+ channels

Many of the above results are consistent with CBD actions on Na^+ channels. We therefore investigated CBD effects on whole-cell Na_V currents in two isolated cell types from different species. First, we determined CBD effects (1 and 10 μM) in human neuroblastoma SH-SY5Y cells. CBD (10 μM) significantly decreased peak whole-cell Na_V current (-24.8 ± 3.4 pA/pF to −5.8 ± 1.0 pA/pF; $p \leq 0.05$; $n = 8$; Fig. 1C and Table 1). In contrast, 1 μM CBD did not affect Na_V peak current (−27.3 ± 3.0 pA/pF *vs* −23.5 ± 2.9 pA/pF; $p > 0.05$; $n = 7$; Table 1). We then assessed CBD effects on isolated mouse cortical neurons. In recordings where 50% external NaCl was replaced with choline chloride, 10 μM CBD significantly reduced peak whole-cell Na_V currents (-95.1 ± 14.4 pA/pF to −27.1 ± 7.1 pA/pF; $p \leq 0.05$; $n = 5$; Fig. 1D and Table 1), a CBD-mediated inhibition of similar magnitude to that seen in SH-SY5Y cells.

Using FMP dye, we assessed the concentration of the Na^+ channel opener veratridine required to produce an EC_{80} response for $hNa_V1.1$ (47 μM), 1.2 (37 μM) and 1.5 (59 μM) (data not shown), before applying these concentrations to cells pre-incubated with CBD or lidocaine. CBD and lidocaine suppressed EC_{80} veratridine-induced fluorescence in recombinant $hNa_V1.1$, 1.2 or 1.5 cells (Fig. 1E and F, Table 1); in control experiments, CBD and lidocaine alone had no effect on fluorescence. CBD's IC_{50} at the three Na_V subtypes was 27–33 μM and the majority of inhibition developed rapidly at 10–30 μM in all cases (Fig. 1F). Lidocaine IC_{50} values were higher (Table 1), but yielded a more stereotypical concentration-response curve (Fig. 1E).

3.3. Effects of CBG on Na^+ channels and on PTZ-induced seizures in rat

We next assessed actions of CBG on Na_V channels. CBG (10 μM) blocked peak Na_V current in SH-SY5Y cells and mouse cortical neurons, (Table 1; Fig. 2A and B). CBG (10 μM) significantly reduced peak Na_V current in SH-SY5Y cells (−30.7 ± 6.3 pA/pF to −7.8 ± 0.5 pA/pF; $p \leq 0.05$; $n = 6$) and mouse cortical neurons (38.0 ± 7.4 pA/pF to −8.0 ± 1.6 pA/pF; $p \leq 0.01$; $n = 8$). CBG-mediated block similar to equimolar CBD effects in these cells. CBG affected EC_{80} veratridine-induced fluorescence (Table 1; CBG $hNa_V1.1$ in Fig. 2C); CBG IC_{50}s for the three Na_V subtypes was 36–88 μM and of the same order of magnitude as CBD in the same cell lines (Table 1).

Finally, we assessed the anticonvulsant potential of CBG (50–200 mg/kg) in the PTZ model of generalised seizures in rats. CBG had no effect on the severity (Fig. 2D), incidence or timing of PTZ-induced seizures, and did not alter mortality (data not shown). Thus, despite showing Na_V channel block, CBG exerted no anticonvulsant actions at any dose.

4. Discussion

We report for the first time that the pCBs, CBD and CBG are relatively low affinity (micromolar) Na_V channel blockers *in vitro* in murine cortical and hippocampal neurons, a human cell line, and stable cell lines expressing $hNa_V1.1$, $hNa_V1.2$ and $hNa_V1.5$ channels. However, whilst CBD is well-documented as an effective anticonvulsant [4–6,19,20], we also show for the first time that CBG has no anticonvulsant properties *in vivo*.

A wide range of pharmacological effects have been ascribed to CBD [2]; however, the molecular target(s) and/or mechanism(s) of action by which CBD suppresses seizures remains unknown. In our

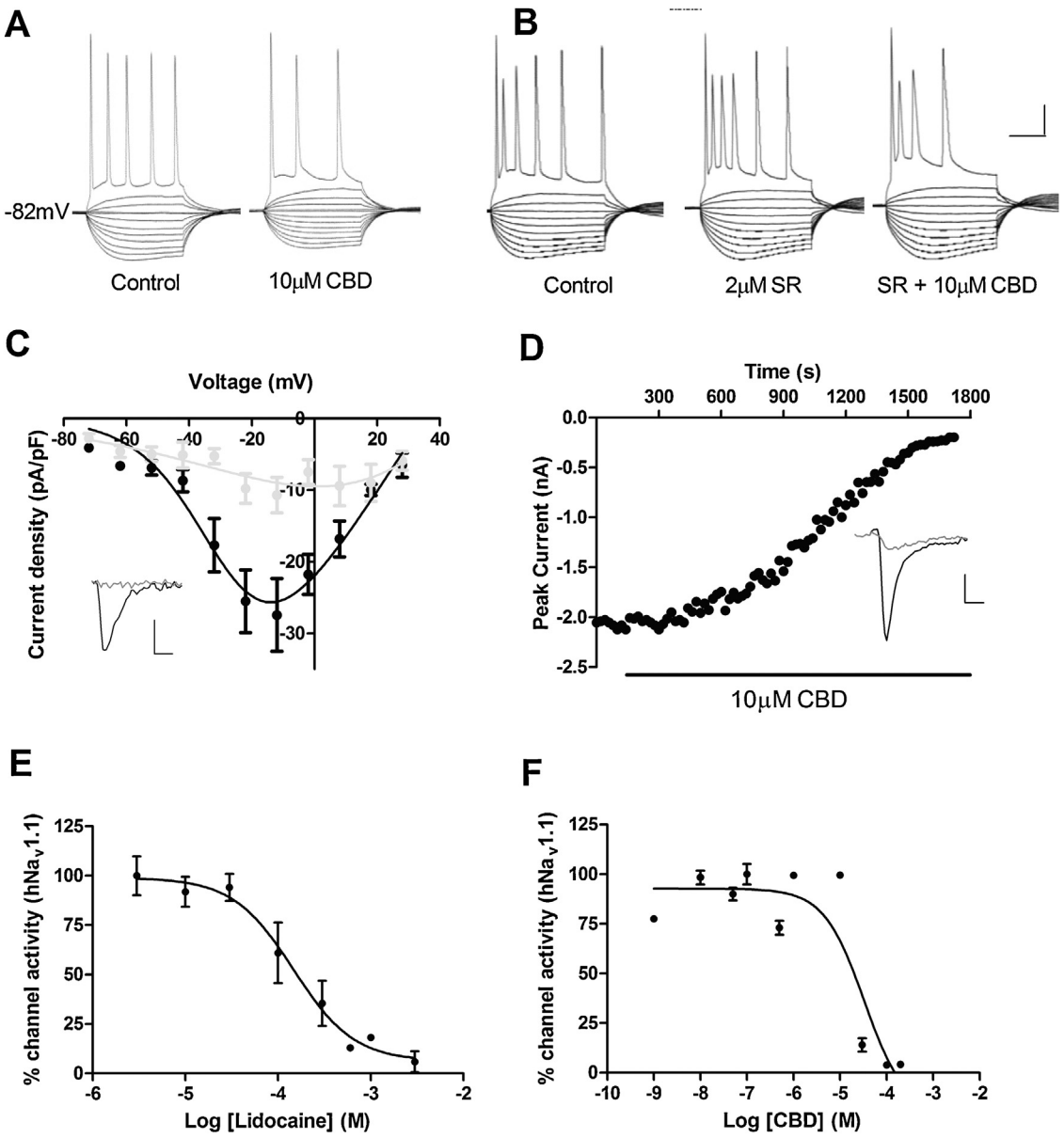

Fig. 1. Effect of cannabidiol (CBD) on Na_V function. (A and B) 10 μM CBD effects on CA1 hippocampal neuron evoked spike firing in brain slices in the absence (A) and presence (B) of 2 μM SR141716A. (C) 10 μM CBD effects on the current-voltage profile of Na_V currents in SH-SY5Y cells ($n = 8$). (D) Representative timecourse of 10 μM CBD effects on peak Na_V current in mouse cortical neurons. (E and F) Effect of lidocaine (E) and CBD (F) on veratridine-induced $hNa_V1.1$ channel activity in CHO cells. Axes in (B) apply to (A) and (B) and represent 20 mV (y) and 50 ms (x) In (C) and (D), control data are shown in black, 10 μM CBD in grey. Insets in (C) and (D) show representative effects of 10 μM CBD at peak Na_V amplitude. Axes in (C) represent 100 pA (y) and 1 ms (x); axes in (D) represent 500 pA (y) and 1 ms (x).

hands, CBD is anticonvulsant at 100 mg/kg i.p. in rat [5,6]; therefore, in the present study, we investigated CBD's effects on Na_V at (1 and 10 μM) concentrations directly relevant to brain levels that cause seizure suppression [14]. Our first assay investigated CBD's effects on non-synaptically evoked neuronal firing. 10 μM CBD significantly affected responses from rat CA1 hippocampal neurons, altering both the membrane resistance and the spike firing. The decrease in membrane resistance could underlie the reduced firing

Table 1
Summary of cannabidiol (CBD) and cannabigerol (CBG) actions on Na^+ channels.

Assay/drug concentration details			Cannabidiol	Cannabigerol	lidocaine
% peak I_{NaV} remaining	SH-SY5Y neuroblastoma	1 μM	86.8 ± 7.1	73.4 ± 5.9*	–
		10 μM	23.8 ± 3.6*	28.6 ± 3.4*	–
	Mouse cortical neurons	10 μM	28 ± 4*	27 ± 6*	–
FLIPR IC_{50} (concentration required to block 50% of ED_{80} veratridine signal)		$hNa_V1.1$	33 ± 0.39 μM	88 ± 1.2 μM	147 ± 0.09 μM
		$hNa_V1.2$	29 ± 0.02 μM	79 ± 0.09 μM	513 ± 0.05 μM
		$hNa_V1.5$	27 ± 0.32 μM	36 ± 0.03 μM	55 ± 0.08 μM

Data given are ±S.E.M. $n = 5–8$ for patch experiments and $n = 3$ replicates for FLIPR data.
* $p \leq 0.05$ *vs* comparator controls.

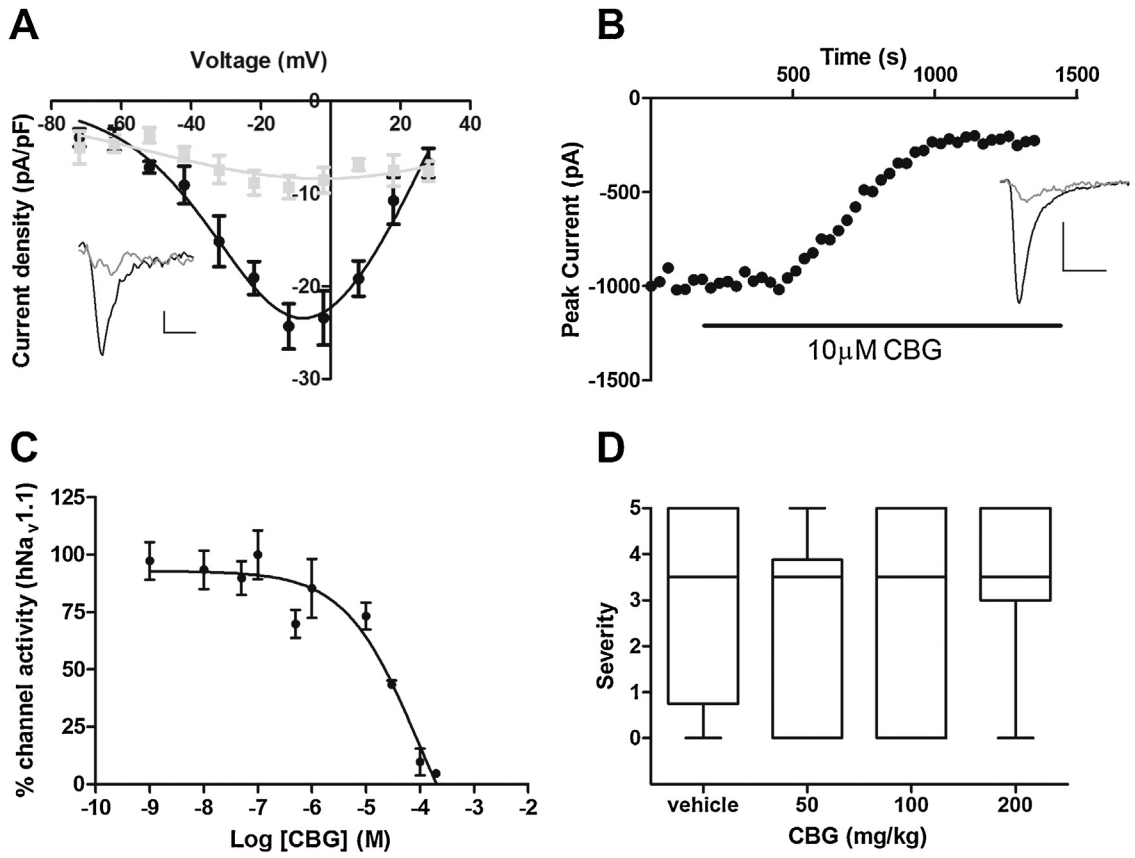

Fig. 2. Effect of cannabigerol (CBG) on PTZ seizures and Na_V function. (A) Effect of CBG (50–200 mg/kg) on seizure severity. Boxes represent 25th and 75th percentiles, error bars represent maxima and minima and horizontal line shows the median. (B) The effect of 10 μM CBG on the current-voltage profile of Na_V currents in SH-SY5Y cells ($n = 6$). (C) Representative timecourse of effect of 10 μM CBG on peak Na_V current in mouse cortical neurons. (D) Effect of CBG on veratridine-induced $hNa_V1.1$ channel activity in CHO cells. Axes in (A) represent 100 pA (y) and 2 ms (x); axes in (B) represent 500 pA (y) and 5 ms (x). In (B) and (C), control data are shown in black, 10 μM CBG in grey. Insets in (C) and (D) show representative effects of 10 μM CBG at peak Na_V amplitude.

frequency and increase in current required to evoke spike firing in the presence of CBD. As this change in steady-state resistance occurred at resting membrane potentials, it could reflect potentiation of leak K^+ channels (*e.g.* TASK and TREK; widely expressed in the hippocampus [21]). However, it is also possible that CBD actions at Na_V channels were responsible for changes in firing frequency. In addition to effects on frequency, CBD increased spike duration, an effect consistent with direct action on Na_V channels. CBD's effects on spike frequency and duration were largely retained in the presence of SR141716A, suggesting they are independent of CB1 function. CBD did not alter membrane resistance in the presence of SR141716A, most likely due to the opposing effect on membrane resistance exerted by SR141716A. The finding that CBD effects on spike frequency and number persisted when CBD and SR141716A were co-administered supports the assertion that they were not solely underpinned by membrane resistance changes.

We found that 10 μM CBD had no effect on spike amplitude, which one might expect to decrease in the presence of a Na_V channel blocker. By contrast, when we investigated the effects of 10 μM CBD on isolated Na_V currents in SH-SY5Y cells and mouse cortical neurons, Na_V amplitude was significantly decreased. It is unlikely that variations in Na_V subtype expression underlie the contrasting results since reported SH-SY5Y subtypes include $Na_V1.2$, 1.3, 1.7 and possibly 1.9 [22,23] whilst rat and mouse neurons express Na_V 1.1, 1.2, 1.3 and 1.6 [24,25]. A more likely possibility is that CBD has additional effects on isolated conductances (*e.g.* activation of concurrent conductances such as voltage-activated K^+ channels or tonic synaptic effects [26]) which could mask effects on spike amplitude in current-clamp recordings from a mixed population of ion channels in rat hippocampal slices.

The fluorescence-based assay of $hNa_V1.1$, 1.2 and 1.5 also showed that CBD blocks specific hNa_V isoforms. These results were notable in two aspects. Firstly, whilst 10 μM CBD blocked ~75% of Na_V current measured in voltage-clamp experiments, this concentration had little/no effect on veratridine-stimulated Na_V activity. Instead, CBD had IC_{50} values of ~30 μM for all three subtypes. A possible explanation lies with the differing approaches used by the electrophysiological and fluorescence-based assays to activate Na_V channels. For the former, direct depolarisation *via* current injection through the patch clamp electrode allows physiologically relevant cycling through open/inactivated/closed Na_V channel states. In the latter case, veratridine holds the Na_V channel open and prevents inactivation by binding at the neurotoxin receptor site 2 and so precludes investigation of state-dependent drug effects. The second notable aspect of the fluorescence data was that CBD-induced inhibition at each hNa_V subtype developed rapidly between 10–30 μM whereas lidocaine exhibited a more stereotypical concentration-response profile. Such a marked response in this concentration range is typical of pCBs in membrane- and cell-based assays [16], although the molecular nature of pCB interactions with membrane-bound target proteins such as ion channels and receptors requires the identification of as yet unknown binding sites.

We show, for the first time, that CBG is not anticonvulsant in the PTZ model of generalised seizures, in contrast to many currently-used AEDs and the pCBs, CBD, cannabidivarin and, to a lesser extent, Δ^9-tetrahydracannabivarin [6,18,27]. Whilst CBG was not effective in the PTZ model, which is tractable to several anticonvulsant

plant cannabinoids, CBG's effects in other models have not yet been explored. The fact that CBD and CBG both act as low affinity (micromolar) Na_V channel blockers allows us to speculate on the role of Na_V blockade in the anticonvulsant effects of different the cannabinoids investigated here, particularly since C_{max} for CBG and CBD are comparable when delivered i.p. in rat [14]. Voltage-clamp experiments in SH-SY5Y and mouse cortical cells indicated that 10 μM CBG exerts a similar blockade of Na_V channels to 10 μM CBD and the only difference between CBD and CBG was observed in fluorescence experiments where CBG IC_{50}s were 2–3 fold higher at $hNa_V1.1$ and 1.2 (but were similar at $hNa_V1.5$). Overall in our study, Na_V blocking actions do not correlate with CBD's anticonvulsant properties and CBG's lack thereof in the PTZ model.

We conclude that cannabinoid-induced Na_V blockade *per se* is not a primary mechanism of anticonvulsant action for CBD. Of particular future interest is a recent clinical survey of the effect of CBD on intractable paediatric epilepsies ([8,28]). Here, CBD is reported to treat patients with Dravet syndrome, an epilepsy that, importantly, is routinely exacerbated by AEDs that block Na_V (*e.g.* carbamazepine; [29]). This data is not only consistent with the proposal that CBD does not suppress seizures *via* Na_V blockade, but fully supports previous findings [5,6] that identify CBD as a genuine treatment option for human epilepsy which is now under clinical investigation [8].

Acknowledgements

The authors thank GW Pharmaceuticals and Otsuka Pharmaceuticals for research sponsorship and provision of pCBs, the University of Reading for research sponsorship to I. Smith and Molecular Devices, Wokingham for access to equipment and expertise.

References

[1] M. Lerner, Marihuana: tetrahydrocannabinol and related compounds, Science 140 (3563) (1963) 175–176.
[2] A.J. Hill, C.M. Williams, B.J. Whalley, G.J. Stephens, Phytocannabinoids as novel therapeutic agents in CNS disorders, Pharmacol. Ther. 133 (1) (2012) 79–97.
[3] A.A. Izzo, F. Borrelli, R. Capasso, V. Di Marzo, R. Mechoulam, Non-psychotropic plant cannabinoids: new therapeutic opportunities from an ancient herb, Trends Pharmacol. Sci. 30 (10) (2009) 515–527.
[4] P. Consroe, A. Wolkin, Cannabidiol–antiepileptic drug comparisons and interactions in experimentally induced seizures in rats, J. Pharmacol. Exp. Ther. 201 (1) (1977) 26–32.
[5] N.A. Jones, S.E. Glyn, S. Akiyama, T.D. Hill, A.J. Hill, S.E. Weston, M.D. Burnett, Y. Yamasaki, G.J. Stephens, B.J. Whalley, C.M. Williams, Cannabidiol exerts anticonvulsant effects in animal models of temporal lobe and partial seizures, Seizure 21 (5) (2012) 344–352.
[6] N.A. Jones, A.J. Hill, I. Smith, S.A. Bevan, C.M. Williams, B.J. Whalley, G.J. Stephens, Cannabidiol displays antiepileptiform and antiseizure properties in vitro and in vivo, J. Pharmacol. Exp. Ther. 332 (2) (2010) 569–577.
[7] J.M. Cunha, E.A. Carlini, A.E. Pereira, O.L. Ramos, C. Pimentel, R. Gagliardi, W.L. Sanvito, N. Lander, R. Mechoulam, Chronic administration of cannabidiol to healthy volunteers and epileptic patients, Pharmacology 21 (3) (1980) 175–185.
[8] GW Pharmaceuticals, GW Pharmaceuticals commences Phase 1 clinical trial of GWP42006 for the treatment of epilepsy, 2013 02/10/2013; available from: http://www.gwpharm.com/Phase1Epilepsy.aspx
[9] H.R. Ross, I. Napier, M. Connor, Inhibition of recombinant human T-type calcium channels by Delta9-tetrahydrocannabinol and cannabidiol, J. Biol. Chem. 283 (23) (2008) 16124–16134.
[10] L. De Petrocellis, A. Ligresti, A.S. Moriello, M. Allara, T. Bisogno, S. Petrosino, C.G. Stott, V. Di Marzo, Effects of cannabinoids and cannabinoid-enriched Cannabis extracts on TRP channels and endocannabinoid metabolic enzymes, Br. J. Pharmacol. 163 (7) (2011) 1479–1494.
[11] L. De Petrocellis, P. Orlando, A.S. Moriello, G. Aviello, C. Stott, A.A. Izzo, V. Di Marzo, Cannabinoid actions at TRPV channels: effects on TRPV3 and TRPV4 and their potential relevance to gastrointestinal inflammation, Acta Physiol. (Oxf.) 204 (2) (2012) 255–266.
[12] N. Qin, M.P. Neeper, Y. Liu, T.L. Hutchinson, M.L. Lubin, C.M. Flores, TRPV2 is activated by cannabidiol and mediates CGRP release in cultured rat dorsal root ganglion neurons, J. Neurosci. 28 (24) (2008) 6231–6238.
[13] S.C. Schachter, Currently available antiepileptic drugs, Neurotherapeutics 4 (1) (2007) 4–11.
[14] S. Deiana, A. Watanabe, Y. Yamasaki, N. Amada, M. Arthur, S. Fleming, H. Woodcock, P. Dorward, B. Pigliacampo, S. Close, B. Platt, G. Riedel, Plasma and brain pharmacokinetic profile of cannabidiol (CBD), cannabidivarine (CBDV), Delta(9)-tetrahydrocannabivarin (THCV) and cannabigerol (CBG) in rats and mice following oral and intraperitoneal administration and CBD action on obsessive-compulsive behaviour, Psychopharmacology (Berl) 219 (3) (2012) 859–873.
[15] J.L. Biedler, L. Helson, B.A. Spengler, Morphology and growth: tumorigenicity, and cytogenetics of human neuroblastoma cells in continuous culture, Cancer Res. 33 (11) (1973) 2643–2652.
[16] M.G. Cascio, L.A. Gauson, L.A. Stevenson, R.A. Ross, R.G. Pertwee, Evidence that the plant cannabinoid cannabigerol is a highly potent alpha2-adrenoceptor agonist and moderately potent 5HT1A receptor antagonist, Br. J. Pharmacol. 159 (1) (2010) 129–141.
[17] J.C. McGrath, G.B. Drummond, E.M. McLachlan, C. Kilkenny, C.L. Wainwright, Guidelines for reporting experiments involving animals: the ARRIVE guidelines, Br. J. Pharmacol. 160 (7) (2010) 1573–1576.
[18] A.J. Hill, S.E. Weston, N.A. Jones, I. Smith, S.A. Bevan, E.M. Williamson, G.J. Stephens, C.M. Williams, B.J. Whalley, Delta(9)-tetrahydrocannabivarin suppresses in vitro epileptiform and in vivo seizure activity in adult rats, Epilepsia 51 (8) (2010) 1522–1532.
[19] P. Consroe, M.A. Benedito, J.R. Leite, E.A. Carlini, R. Mechoulam, Effects of cannabidiol on behavioral seizures caused by convulsant drugs or current in mice, Eur. J. Pharmacol. 83 (3–4) (1982) 293–298.
[20] M.J. Wallace, J.L. Wiley, B.R. Martin, R.J. DeLorenzo, Assessment of the role of CB1 receptors in cannabinoid anticonvulsant effects, Eur. J. Pharmacol. 428 (1) (2001) 51–57.
[21] E.M. Talley, G. Solorzano, Q. Lei, D. Kim, D.A. Bayliss, Cns distribution of members of the two-pore-domain (KCNK) potassium channel family, J. Neurosci. 21 (19) (2001) 7491–7505.
[22] R. Blum, K.W. Kafitz, A. Konnerth, Neurotrophin-evoked depolarization requires the sodium channel Na(V)1.9, Nature 419 (6908) (2002) 687–693.
[23] I. Vetter, C.A. Mozar, T. Durek, J.S. Wingerd, P.F. Alewood, M.J. Christie, R.J. Lewis, Characterisation of Na(v) types endogenously expressed in human SH-SY5Y neuroblastoma cells, Biochem. Pharmacol. 83 (11) (2012) 1562–1571.
[24] H. Blumenfeld, A. Lampert, J.P. Klein, J. Mission, M.C. Chen, M. Rivera, S. Dib-Hajj, A.R. Brennan, B.C. Hains, S.G. Waxman, Role of hippocampal sodium channel Nav1.6 in kindling epileptogenesis, Epilepsia 50 (1) (2009) 44–55.
[25] W.A. Catterall, A.L. Goldin, S.G. Waxman, International Union of Pharmacology XLVII. Nomenclature and structure-function relationships of voltage-gated sodium channels, Pharmacol. Rev. 57 (4) (2005) 397–409.
[26] C.J. Ledgerwood, S.M. Greenwood, R.R. Brett, J.A. Pratt, T.J. Bushell, Cannabidiol inhibits synaptic transmission in rat hippocampal cultures and slices via multiple receptor pathways, Br. J. Pharmacol. 162 (1) (2011) 286–294.
[27] A.J. Hill, M.S. Mercier, T.D. Hill, S.E. Glyn, N.A. Jones, Y. Yamasaki, T. Futamura, M. Duncan, C.G. Stott, G.J. Stephens, C.M. Williams, B.J. Whalley, Cannabidivarin is anticonvulsant in mouse and rat, Br. J. Pharmacol. 167 (8) (2012) 1629–1642.
[28] B.E. Porter, C. Jacobson, Report of a parent survey of cannabidiol-enriched cannabis use in pediatric treatment-resistant epilepsy, Epilepsy Behav. 29 (3) (2013) 574–577.
[29] C. Chiron, Current therapeutic procedures in Dravet syndrome, Dev. Med. Child Neurol. 53 (Suppl. 2) (2011) 16–18.