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RESEARCH PAPER

Cannabidivarin is anticonvulsant in mouse and rat

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BACKGROUND AND PURPOSE

Phytocannabinoids in *Cannabis sativa* have diverse pharmacological targets extending beyond cannabinoid receptors and several exert notable anticonvulsant effects. For the first time, we investigated the anticonvulsant profile of the phytocannabinoid cannabidivarin (CBDV) *in vitro* and in *in vivo* seizure models.

EXPERIMENTAL APPROACH

The effect of CBDV (1–100 μM) on epileptiform local field potentials (LFPs) induced in rat hippocampal brain slices by 4-aminopyridine (4-AP) application or Mg^{2+}-free conditions was assessed by *in vitro* multi-electrode array recordings. Additionally, the anticonvulsant profile of CBDV (50–200 mg·kg^{-1}) *in vivo* was investigated in four rodent seizure models: maximal electroshock (mES) and audiogenic seizures in mice, and pentylenetetrazole (PTZ) and pilocarpine-induced seizures in rats. The effects of CBDV in combination with commonly used antiepileptic drugs on rat seizures were investigated. Finally, the motor side effect profile of CBDV was investigated using static beam and grip strength assays.

KEY RESULTS

CBDV significantly attenuated *status epilepticus*-like epileptiform LFPs induced by 4-AP and Mg^{2+}-free conditions. CBDV had significant anticonvulsant effects on the mES (≥100 mg·kg^{-1}), audiogenic (≥50 mg·kg^{-1}) and PTZ-induced seizures (≥100 mg·kg^{-1}). CBDV (200 mg·kg^{-1}) alone had no effect against pilocarpine-induced seizures, but significantly attenuated these seizures when administered with valproate or phenobarbital at this dose. CBDV had no effect on motor function.

CONCLUSIONS AND IMPLICATIONS

These results indicate that CBDV is an effective anticonvulsant in a broad range of seizure models. Also it did not significantly affect normal motor function and, therefore, merits further investigation as a novel anti-epileptic in chronic epilepsy models.

LINKED ARTICLES

This article is part of a themed section on Cannabinoids. To view the other articles in this section visit http://dx.doi.org/10.1111/bph.2012.167.issue-8

Abbreviations

AED, antiepileptic drugs; 4-AP, 4-aminopyridine; CBD, cannabidiol; CBDV, cannabidivarin; DG, dentate gyrus; ESM, ethosuximide; LFP, local field potential; MEA, multi-electrode array; mES, maximal electroshock; PTZ, pentylenetetrazole; Δ^9-THC, Δ^9-tetrahydrocannabinol; TRP, transient receptor potential; VPA, valproate

Introduction

Epilepsy is a CNS disorder affecting ~1% of the global population, and is symptomatically characterized by chronic, recurrent seizures. A range of treatments are available, although there is still a need for more effective and better-tolerated antiepileptic drugs (AEDs) as illustrated by the pharmacological intractability of ~30% of cases and the poor side effect profile of currently available AEDs (Kwan and Brodie, 2007). *Cannabis sativa* has a long history of use for the control of human seizures (O'Shaughnessy, 1843; Mechoulam, 1986), and is legally used for this in some countries (Sirven and Berg, 2004).

There are >100 phytocannabinoids present in *C. sativa*, of which Δ^9-tetrahydrocannabinol (Δ^9-THC) is the most abundant (Elsohly and Slade, 2005; Mehmedic *et al.*, 2010) and, via partial agonism of the CB_1 cannabinoid receptor, is responsible for the classical psychoactive effects of cannabis (Pertwee, 2008). Although CB_1 cannabinoid receptor agonism can exert anticonvulsant effects in *in vitro* and *in vivo* models (Chesher and Jackson, 1974; Wallace *et al.*, 2001; 2003; Deshpande *et al.*, 2007), the most promising non-psychoactive anticonvulsant phytocannabinoid investigated to date is cannabidiol (CBD), which exerts anticonvulsant actions via an, as yet unknown, non-CB_1 cannabinoid receptor mechanism(s) in animal models *in vitro*, *in vivo* and in humans (Cunha *et al.*, 1980; Consroe *et al.*, 1982; Wallace *et al.*, 2001; Jones *et al.*, 2010); CBD's notable anticonvulsant properties led us to investigate the anticonvulsant potential of its propyl analogue, cannabidivarin (CBDV).

CBDV was first isolated in 1969 (Vollner *et al.*, 1969). At present, little is known about the pharmacological properties of CBDV (Izzo *et al.*, 2009), although Scutt and Williamson reported that CBDV acts via CB_2 cannabinoid receptor-dependent mechanisms (Scutt and Williamson, 2007). More recently, De Petrocellis and co-workers reported differential CBDV effects at transient receptor potential (TRP) channels *in vitro*, where it acted as a human TRPA1, TRPV1 and TRPV2 agonist (EC_{50} values: 0.42, 3.6 and 7.3 μM, respectively) and a TRPM8 antagonist (IC_{50}: 0.90 μM) (De Petrocellis *et al.*, 2011a,b). Additionally, CBDV has been shown to inhibit the primary synthetic enzyme of the endocannabinoid, 2-arachidonoylglycerol (Bisogno *et al.*, 2003), diacylglycerol lipase α (IC_{50} 16.6 μM) *in vitro* (De Petrocellis *et al.*, 2011a). While the pharmacological relevance of these effects has not been confirmed *in vivo*, they further illustrate the diversity of non-Δ^9-THC phytocannabinoid pharmacology and support the emergent role of multiple non-CB receptor targets (Pertwee, 2010; Hill *et al.*, 2012).

Here, we identified anticonvulsant effects of CBDV for the first time; CBDV suppressed *in vitro* epileptiform activity in brain slices and acted as an anticonvulsant *in vivo*. However, normal motor function was not significantly affected by CBDV, therefore, further investigations into the clinical development of CBDV as a novel AED are warranted.

Methods

In vitro *electrophysiology*

Tissue preparation. All studies involving animals are reported in accordance with the ARRIVE guidelines for reporting experiments involving animals (Kilkenny *et al.*, 2010; McGrath *et al.*, 2010) and all experiments were carried out in accordance with Home Office regulations [Animals (Scientific Procedures) Act, 1986]. Transverse hippocampal slices (~450-μm thick) for multi-electrode array (MEA) recordings were prepared from female and male adult Wistar Kyoto rats (P > 21; Harlan, Bicester, UK) using a Vibroslice 725 M (Campden Instruments Ltd., Loughborough, UK) as previously described (Jones *et al.*, 2010).

MEA recordings. MEA recordings and analyses were conducted as described in Hill *et al.* (2010). Once established [by addition of either 100 μM 4-aminopyridine (4-AP) or omission of $MgSO_4.7H_2O$ without substitution], epileptiform activity was permitted to continue for 30 min (control bursting) before sequential addition of 1, 10 and 100 μM CBDV (30 min each). Epileptiform activity was characterized by spontaneous local field potentials (LFPs) recorded simultaneously from 59 electrodes covering the majority of the hippocampal slice preparation. The amplitude and duration of epileptiform LFPs were analysed for each electrode. Data from individual electrodes, based on their position in each hippocampal subregion, were pooled to provide mean results for each subregion across $n \geq 5$ slices from $n \geq 5$ animals per model. Matlab 6.5 and 7.0.4 (Mathworks, Natick, MA, USA), Microsoft Excel (Microsoft, Redmond, WA, USA), MC_DataTool and MC_Rack (Multi Channel Systems GmbH, Reutlingen, Germany) were used to process and present data as described in Hill *et al.* (2010). Inherent changes in LFP amplitude and frequency were corrected for, as described previously (Hill *et al.*, 2010). For reference, the extent of amplitude rundown correction applied is illustrated in Figure 1C and D. LFP frequency was calculated per slice ($n \geq 5$ for each model) and represents the number of LFP bursts per unit time. Examples of single bursts from each model can be seen in Figure 1A and B. Drug-induced changes in burst duration, amplitude and frequency are expressed as normalized proportions of control values ± SEM, corrected where necessary, and were analysed by Wilcoxon's paired test with Holm's sequential Bonferroni correction.

In vivo *seizure models*

Animals. In all cases before seizure induction, animals were maintained on a 12 h light/dark cycle with free access to food and water (with the exception of rats that received oral CBDV, see later). Audiogenic seizure experiments with dilute, brown, non-Agouti (DBA/2) mice (3–4 weeks old; Elevage Janvier, Le Genest-Saint-Isle, France) were performed at Porsolt Research Laboratory (Le Genest-Saint-Isle, France) in accordance with French legislation and under licence from the French Ministry for Agriculture and Fisheries. mES experiments with ICR (CD-1) mice (5 weeks old; SLC Japan Inc., Shizuoka, Japan) were performed at Otsuka Pharmaceuticals Co, Ltd. (Tokushima, Japan) in accordance with the guidelines of the Physiological Society of Japan. In total, 80 mice were used. Seizure studies in male Wistar Kyoto rats (Harlan, 3–4 weeks old; in total, 640 rats were used) were performed at the University of Reading, UK; all experiments were carried out in accordance with UK Home Office regulations [Animals (Scientific Procedures) Act 1986].

CBDV administration. CBDV (50, 100 or 200 $mg \cdot kg^{-1}$; GW Pharmaceuticals Ltd., Salisbury, UK) in an ethanol : Cremo-

Table 1

Seizure behaviour scoring scales for PTZ and pilocarpine-induced seizures

Score	PTZ-induced seizures	Pilocarpine-induced seizures
0	Normal behaviour	Normal behaviour
1	Isolated myoclonic jerks	Mouth clonus
2	Atypical clonic seizure	Unilateral forelimb clonus
3	Fully developed bilateral forelimb clonus	Bilateral forelimb clonus
3.5	Forelimb clonus with tonic component and body twist	NA
4	Tonic–clonic seizure with suppressed tonic phase*	Bilateral forelimb clonus with rearing and falling
4.5	NA	Tonic–clonic seizure with postural control retained
5	Fully developed tonic–clonic seizure*	Tonic–clonic seizure*

Seizure severity scoring scales are shown for each model, although no equivalency of severity should be assumed between scales for different models.
*Indicates a loss of righting reflex.
NA = not applicable.

phor: saline (0.9% w v^{-1} NaCl vehicle; 2:1:17; all Sigma, Poole, UK) was administered by an i.p. injection 1 h before seizure induction in all the models, with the exception of mES where it was administered 30 min before seizure induction. All experiments included a control group, which received volume-matched vehicle, against which other groups were assessed. In mice experiments, n = 10 per group and in rat, n = 15 per group. In experiments where CBDV was administered p.o. (gavage), 400 mg·kg^{-1} CBDV or volume-matched vehicle [20% solutol (Sigma) in 0.9% w v^{-1} NaCl] was administered after the animals had been deprived of food for 13.5 h and 3.5 h before i.p. administration of pentylenetetrazole (PTZ), n = 15 for both groups (see Supporting Information Appendix S1 for details on oral dose levels).

Seizure induction. mES seizures were induced in mice by a stimulator (Ugo Basile ECT, Comerio, Italy) via earlap clamps at a current of 30 mA delivered at 100 Hz for 200 ms. DBA/2 mice were placed in a Plexiglas jar 1 h after CBDV/vehicle administration. A mounted bell (110–120 dB) was activated until occurrence of a tonic audiogenic seizure or for a maximum of 60 s. To induce generalized seizures in rats, 85 mg·kg^{-1} PTZ was injected i.p. *Status epilepticus* with a temporal lobe focus was induced in rats by injecting pilocarpine hydrochloride (Sigma; in 0.9% w v^{-1} NaCl) 380 mg·kg^{-1} i.p., 45 min after pretreating the rats with methylscopolamine (Sigma; in 0.9% w v^{-1} NaCl) 1 mg·kg^{-1} i.p., which blocks the peripheral effects of pilocarpine.

Seizure analysis. In mES experiments, mice were observed for 10 s during electroshock, tonic hindlimb extension occurrence was noted and expressed as a percentage of the total number of animals for each group. Audiogenic seizure behaviour was observed visually, while rat seizures were video recorded (Farrimond *et al.*, 2009). For audiogenic seizures, the incidence (as a percentage) of the most severe (tonic–clonic) seizures, mortality and seizure-free animals were calculated for each group. These parameters, as well as seizure duration and severity, were also determined for rat seizures. Rat behaviour was coded blind offline using The Observer Pro software (Noldus, Wageningen, The Netherlands) and seizure severity scales appropriate to each seizure type (Table 1). Values are expressed as mean ± SEM throughout.

Co-administration experiments. The effect of co-administration of clinically-used AEDs with 200 mg·kg^{-1} CBDV on PTZ- and pilocarpine-induced seizures was investigated. For details, see Supporting Information Appendix S1. Briefly, in each experiment, an AED was administered i.p., at either ~20, ~40 or ~70% maximal effective dose, in the absence or presence of 200 mg·kg^{-1} CBDV (n = 15 per group, 120 per experiment); the convulsant (PTZ or pilocarpine) was administered 1 h after CBDV or its vehicle. The experimental design is illustrated and summarized in Table 2. In the PTZ model, CBDV was co-administered with valproate (VPA) or ethosuximide (ESM) before PTZ, and with VPA or phenobarbital (PB) before pilocarpine. These AEDs were chosen based on their clinical profile and their reported efficacy in the models used here, with VPA suppressing both seizure types and ESM and phenobarbital suppressing PTZ and pilocarpine respectively (Loscher *et al.*, 1991; Sofia *et al.*, 1993; Shantilal *et al.*, 1999; Lindekens *et al.*, 2000; Loscher, 2011). In co-administration experiments, seven (2.9%) rats exhibited a fatal reaction to CBDV administration. Behaviourally, this manifested as rapid development (within 300 s) of lethargic convulsive movements followed by death. Overall, across all PTZ and pilocarpine experiments, this effect was seen in 2.6% of all rats that received 200 mg·kg^{-1} CBDV, but not at all in side effect tests. No adverse effects of other CBDV doses were observed in rats, and none at any dose in mice. The animals that died were omitted from all analyses.

Statistics. In experiments where i.p. CBDV alone was administered, the effects of CBDV on seizure severity, onset latency and seizure duration were assessed by one-way ANOVA with *post hoc* Tukey's tests as appropriate. Chi-squared tests followed by *post hoc* Fisher's exact tests were used where appro-

Table 2

Experimental design and time course of co-administration experiments

		60 min			
CBDV/vehicle treatment (i.p.)		Time A (min)	AED treatment (i.p.)	Time B (min)	Seizure induction and recording
PTZ experiments	200 mg·kg^{-1} CBDV (n = 60)	30	VPA vehicle, 50, 100, 250 mg·kg^{-1} VPA (n = 15 each)	30	85 mg·kg^{-1} PTZ 30-min recording
	CBDV vehicle (n = 60)		VPA vehicle, 50, 100, 250 mg·kg^{-1} VPA (n = 15 each)		
	200 mg·kg^{-1} CBDV (n = 60)	30	ESM vehicle, 60, 120, 175 mg·kg^{-1} ESM (n = 15 each)	30	
	CBDV vehicle (n = 60)		ESM vehicle, 60, 120, 175 mg·kg^{-1} ESM (n = 15 each)		
Pilocarpine experiments	200 mg·kg^{-1} CBDV (n = 60)	15	VPA vehicle, 62.5, 125, 250 mg·kg^{-1} VPA (n = 15 each)	45	380 mg·kg^{-1} pilocarpine 60-min recording
	CBDV vehicle (n = 60)		VPA vehicle, 62.5, 125, 250 mg·kg^{-1} VPA (n = 15 each)		
	200 mg·kg^{-1} CBDV (n = 60)	15	PB vehicle, 10, 20, 40 mg·kg^{-1} PB (n = 15 each)	45	
	CBDV vehicle (n = 60)		PB vehicle, 10, 20, 40 mg·kg^{-1} PB (n = 15 each)		

'Time A' column: time between CBDV/CBDV vehicle and AED administration. 'Time B' column: time between AED/vehicle and convulsant. The duration of the seizure recording is indicated in the final column. PB, phenobarbital, VPA, valproate, ESM, ethosuximide.

priate to assess differences in incidence parameters. Where CBDV was co-administered with an AED, two-way ANOVA or log-linear modelling was used to analyse the effects of CBDV and AEDs. Log-linear modelling was used to model the interactions between drug co-administration and incidence parameters (e.g. mortality, % seizure-free). If the model indicated a significant effect of drug treatment, further analysis to determine the contribution of CBDV, the relevant AED and any drug × drug interaction was performed; these analyses are given in the text and in Supporting Information Tables S1 and S2.

Motor function assays

The effects of CBDV (50, 100 and 200 mg·kg^{-1}) and VPA (125, 250 and 350 mg·kg^{-1}) on normal rat motor function were assessed on a 1 m raised static beam and by a grip strength test (see Supporting Information Appendix S1 for details).

All receptor and ion channel nomenclature conforms to BJP's *Guide to Receptors and Channels* (Alexander *et al.*, 2011).

Results

Effects of pure CBDV in the Mg^{2+}-free and 4-AP in vitro models of epileptiform activity

The effects of CBDV (1–100 μM) on epileptiform activity, induced by Mg^{2+}-free aCSF (Figure 1A) or 100 μM 4-AP (Figure 1B), in rat acute hippocampal slices were examined. CBDV significantly decreased the amplitude and duration of epileptiform LFPs induced by Mg^{2+}-free aCSF (Figure 1C and D; $P \leq 0.05$); significant effects were seen at ≥10 μM, and the CA3 region was more resistant to the effects of CBDV than the dentate gyrus (DG) or CA1 (Figure 1C and D). Conversely, CBDV significantly increased Mg^{2+}-free-induced LFP frequency (≥10 μM; Figure 1E; $P \leq 0.05$).

An anti-epileptiform effect of 100 μM CBDV on the amplitude of 4-AP-induced epileptiform LFPs was observed in the CA1 region alone (Figure 1F; $P \leq 0.05$), whereas LFP duration was significantly lowered in all hippocampal regions by ≥10 μM CBDV (Figure 1G) and, by contrast to the Mg^{2+}-free model, 4-AP-induced LFP frequency was significantly decreased by all CBDV concentrations tested (Figure 1H; $P \leq 0.05$). Thus, CBDV attenuated the duration of amplitude of LFPs in both models, and had differential effects on frequency.

Effects of CBDV on maximal electroshock (mES) and audiogenic seizures in mice

The effects of CBDV (50–200 mg·kg^{-1}) on mES convulsions and audiogenic seizures in mice were investigated. CBDV had a significant anticonvulsant effect on animals displaying tonic hindlimb extension after mES [$\chi^2(3) = 15.000$; $P \leq 0.001$; Figure 2A]; significantly fewer animals that received 100 or 200 mg·kg^{-1} CBDV exhibited hindlimb extension (both groups 30%) than those that received vehicle (90%, Figure 2A; $P \leq 0.001$ vs. vehicle-treated group). Audiogenic seizures were also significantly attenuated by CBDV (Figure 2B–D). The incidence of tonic convulsions was significantly lower after CBDV administration [$\chi^2(3) = 19.436$, $P \leq 0.001$; Figure 2B]; 80% of vehicle-treated animals developed tonic convulsions compared with only 20% (50 mg·kg^{-1} CBDV), 10% (100 mg·kg^{-1} CBDV) and 0% (200 mg·kg^{-1} CBDV) after drug treatment (each $P \leq 0.001$ *vs* vehicle). The percentage of animals that remained seizure-free was significantly higher after administration of 200 mg·kg^{-1} CBDV (90%) than vehicle [0%; $\chi^2(3) = 27.461$, $P \leq 0.001$; Figure 2C]. Finally, a statistical trend was observed for the mortality rate [$\chi^2(3) = 6.667$, $P \leq 0.1$], with lower mortality after 100 and 200 mg·kg^{-1} CBDV treatment than vehicle (0% vs 30%, respectively; Figure 2D). Thus, CBDV exhibits strong and significant anticonvulsant effects in two broad-screen mouse

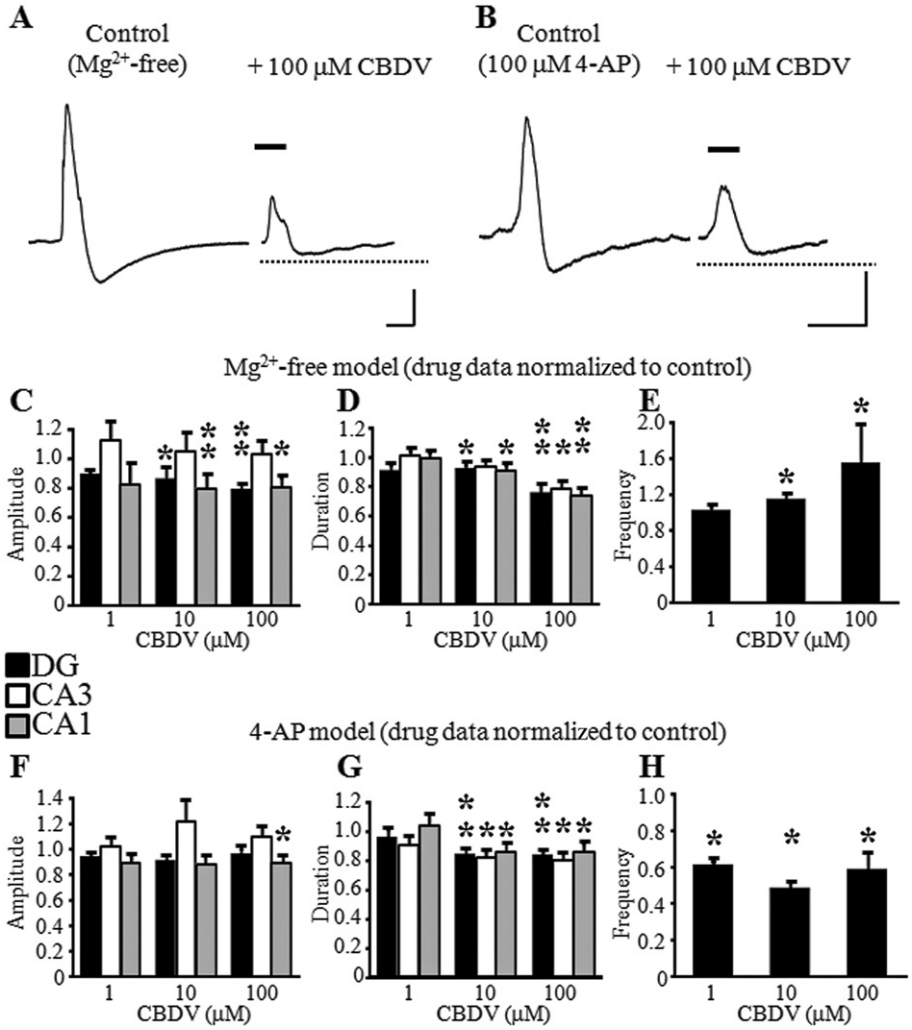

Figure 1

Effects of CBDV on hippocampal epileptiform activity. (A and B) Example traces showing effects of 100 μM CBDV on epileptiform LFPs induced by Mg^{2+}-free conditions (A) or 100 μM 4-AP (B) in the CA1 region. The black bar represents amplitude as corrected for inherent rundown (see Methods); the dotted line below represents control burst duration. Scale in (A): 100 μV/200 ms; (B): 150 μV/200 ms. (C–H) Effects of CBDV on amplitude (C and F), duration (D and G) and frequency (E and H) of epileptiform LFPs induced by Mg^{2+}-free conditions (C–E) or 100 μM 4-AP (F–H). Data are presented as mean ± SEM normalized to control (pre-drug) conditions and corrected for background changes where appropriate (see Methods). LFP amplitude and duration values are expressed for each hippocampal region as in the key. $n = 9–12$. *$P \leq 0.05$, **$P \leq 0.01$ and ***$P \leq 0.001$.

seizure models. Next, we investigated the anticonvulsant potential of CBDV in two further models of seizure in rat that emulate more specific seizure types.

Effects of CBDV on PTZ- and pilocarpine-induced seizures in rats

CBDV significantly decreased PTZ seizure severity ($F_{3,58}$ = 4.423, $P \leq 0.05$; Figure 3A); the median seizure severity after vehicle administration was tonic–clonic convulsion score 5, but after 200 mg·kg^{-1} CBDV administration seizure severity was significantly lowered to a median severity of bilateral clonic convulsion score 3 ($P \leq 0.05$). CBDV also significantly reduced mortality ($\chi^2(3) = 10.356$, $P \leq 0.05$; Figure 3B) at 100 and 200 mg·kg^{-1} CBDV ($P \leq 0.01$). The percentage of animals that remained seizure-free was significantly increased by CBDV administration [$\chi^2(3) = 7.809$, $P \leq 0.05$; Figure 3C]; 33.3% of animals that received 200 mg·kg^{-1} CBDV exhibited no signs of seizure compared with only 6.7% of animals that received vehicle ($P \leq 0.01$). Furthermore, seizure onset was significantly delayed by CBDV treatment ($F_{3,50}$ = 2.971, $P \leq 0.05$; Figure 3D); mean onset latency was significantly longer after administration of 200 mg·kg^{-1} CBDV than vehicle (65 ± 11 s and 40 ± 4 s, respectively; $P \leq 0.05$). Thus, CBDV, administered alone, exhibited strong and significant anticonvulsant effects on PTZ seizures at 200 mg·kg^{-1} (Figure 3A–D) with more limited, but significant, effects at 100 mg·kg^{-1} (Figure 3B).

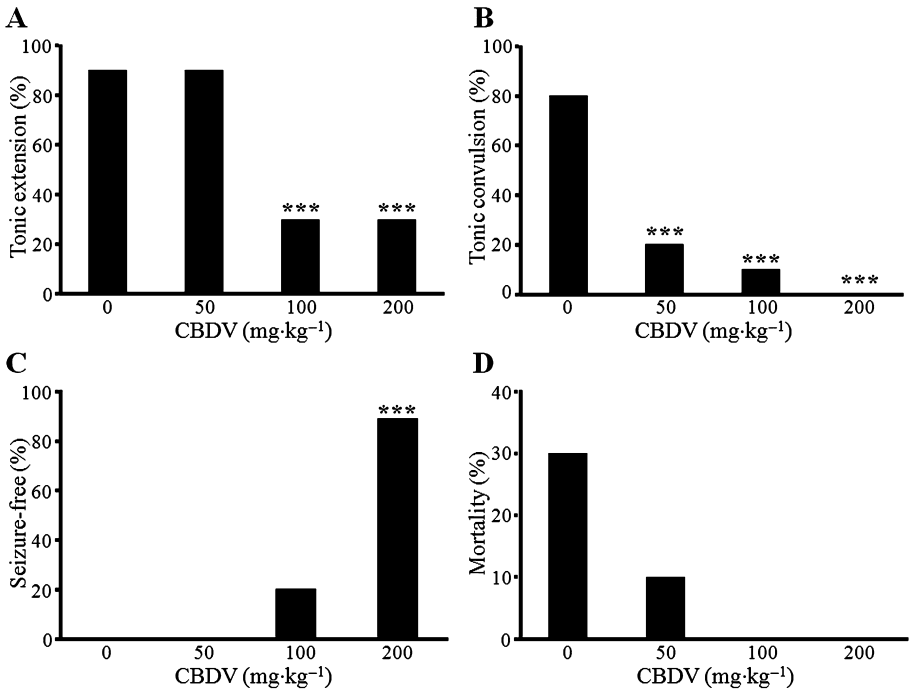

Figure 2

Effects of CBDV on mES and audiogenic seizures in mice. (A) The effect of CBDV on the percentage of animals that exhibited tonic hindlimb extension in response to mES. (B–D) The effect of CBDV (50–200 mg·kg^{-1}) on the percentage of animals that displayed tonic convulsions (B), remained seizure-free (C) or suffered mortality (D) as a result of audiogenic seizure induction. n = 10 in all cases, ***$P \leq 0.001$.

We extended our studies to investigate the effects of CBDV (50–200 mg·kg^{-1}) on the convulsions associated with pilocarpine-induced *status epilepticus* (380 mg·kg^{-1}). CBDV (50–200 mg·kg^{-1}) had no significant effect on the severity ($F_{3,59}$ = 0.049, P > 0.1; Figure 3E) or resultant mortality of pilocarpine convulsions [$\chi^2(3)$ = 1.779, P > 0.1; Figure 3F]. Similarly, CBDV did not significantly affect the percentage of animals that remained seizure-free [$\chi^2(3)$ = 0.110, P > 0.1; Figure 3G] or the latency to the onset of convulsions ($F_{3,53}$ = 0.404, P > 0.1; Figure 3H).

Effect of co-administration of CBDV and AEDs on PTZ- and pilocarpine-induced seizures in rats

We investigated the effects of CBDV when co-administered with AEDs before PTZ or pilocarpine treatment. The effects of combined drug treatment (CBDV + AED) on seizure parameters are illustrated in Figures 4 and 5, as is the contribution of CBDV to these effects. The contribution of AEDs is illustrated in Figures 4 and 5 while statistical analyses of AED effects and any interaction between CBDV and AEDs are shown in Supporting Information Tables S1 and S2.

CBDV 200 mg·kg^{-1} was co-administered with VPA (50–250 mg·kg^{-1}) or ESM (60–175 mg·kg^{-1}). In the CBDV + VPA experiments, drug co-administration had significant anticonvulsant effects on all seizure parameters except the percentage of animals remaining seizure-free. CBDV and VPA co-administration significantly decreased seizure severity ($F_{7,112}$ = 10.449, $P \leq 0.001$; Figure 4A). When modelled by log-linear analyses, our data indicated that drug co-administration decreased mortality (Figure 4B) and the incidence of the most severe (tonic–clonic) seizures (Figure 4C). Seizure onset was significantly delayed by drug co-administration ($F_{7,109}$ = 13.285, $P \leq 0.001$; Figure 4D) and the mean duration of seizures was increased ($F_{7,103}$ = 5.250, $P \leq 0.001$). VPA contributed significantly to all these effects (Figure 4A–D, Supporting Information Table S1). CBDV significantly contributed to the overall decrease in severity ($F_{1,112}$ = 5.748, $P \leq 0.05$; Figure 4A) and mortality [$\chi^2(1)$ = 6.639, $P \leq 0.01$; Figure 4B] and the increase in onset latency ($F_{1,109}$ = 7.393, $P \leq 0.01$; Figure 4C). CBDV did not significantly affect tonic–clonic seizure incidence (Figure 4D) or seizure duration (P > 0.1). No effect of drug treatment on the number of seizure-free animals was observed [$X^2(14)$ = 8.930, P > 0.1] and no significant positive or negative interactions between the effects of 200 mg·kg^{-1} CBDV and VPA were observed (Supporting Information Tables S1, P > 0.1).

Co-administration of 200 mg·kg^{-1} CBDV and ESM (60–175 mg·kg^{-1}) had significant anticonvulsant effects on all parameters of PTZ-induced seizures: CBDV and ESM co-administration significantly decreased seizure severity ($F_{7,110}$ = 12.556, $P \leq 0.001$; Figure 4E), when modelled with log-linear analysis, our data indicated that co-administration also decreased mortality (Figure 4F) and the incidence of the most severe seizures (Figure 4G). Seizure onset latency was significantly increased ($F_{7,76}$ = 7.885, $P \leq 0.001$; Figure 4H), as was the percentage of animals that remained seizure-free (log-linear model; Figure 4I); seizure duration was also significantly decreased ($F_{7,102}$ = 6.934, $P \leq 0.001$). ESM significantly

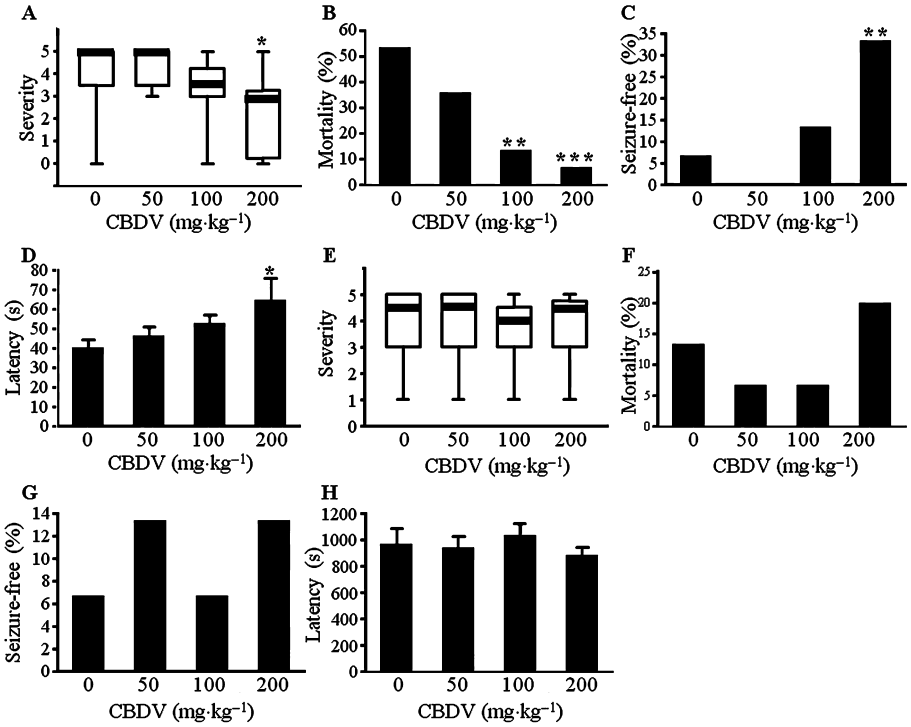

Figure 3

Effects of CBDV on PTZ- and pilocarpine-induced seizures in rats. (A–D) The effect of CBDV on PTZ-induced seizures: seizure severity (A), mortality (B), the proportion of animals remaining seizure-free (C) and the onset latency (D). (E–H) The effect of CBDV on pilocarpine-induced convulsions: severity (E), mortality (F), the percentage of animals remaining seizure-free (G) and the onset latency (H). In (D and H), onset latency is presented as mean ± SEM In (A and E), median severity is represented by a thick horizontal line, the 25th and the 75th percentiles by the box and maxima and minima are represented by 'whiskers'. n = 15 in all cases. *$P \leq 0.05$, **$P \leq 0.01$ and ***$P \leq 0.001$.

contributed to all anticonvulsant effects (Figure 4E-I; Supporting Information Table S1). CBDV contributed significantly to the overall decreases in seizure severity ($F_{1,112}$ = 7.474, $P \leq 0.01$; Figure 4E) and mortality [$\chi^2(1) = 5.174$, $P \leq 0.05$; Figure 4F]; the contribution of CBDV to the increase in onset latency showed a statistical trend ($F_{1,76} = 2.791$, $P \leq 0.1$; Figure 4H). CBDV did not significantly contribute to the effects on seizure duration, the proportion of animals that remained seizure-free (both $P > 0.1$) or the incidence of the most severe seizures ($P > 0.1$; Figure 4G). No significant positive or negative interactions between the effects of 200 mg·kg^{-1} CBDV and ESM were observed (Supporting Information Tables S1, $P > 0.1$).

We next investigated whether 200 mg·kg^{-1} CBDV affected the anticonvulsant actions of VPA or phenobarbital on pilocarpine-induced convulsions. Interestingly, these co-administration experiments highlighted significant anticonvulsant effects of 200 mg·kg^{-1} CBDV not previously observed when CBDV was administered alone. Co-administration of VPA (50–250 mg·kg^{-1}) with 200 mg·kg^{-1} CBDV had significant anticonvulsant effects on all the parameters except the percentage of animals that remained convulsion-free: CBDV and VPA co-administration significantly decreased severity ($F_{7,100}$ = 16.477, $P \leq 0.001$; Figure 5A); when modelled by log-linear analysis, our data indicated that mortality (Figure 5B) and the incidence of the most severe (tonic–clonic) convulsions (Figure 5C) were also decreased by drug co-administration; onset latency was significantly increased ($F_{7,105}$ = 8.649, $P \leq 0.001$; Figure 5D). VPA contributed significantly to all anticonvulsant effects (Figure 5A-D, Supporting Information Table S2) with the interesting exception of mortality. Mortality was higher (but not significantly so) when 62.5 and 125 mg·kg^{-1} VPA were co-administered with vehicle (Figure 5B); however, CBDV had an anticonvulsant effect, significantly decreasing mortality compared with administration of its vehicle [$\chi^2(1) = 4.010$, $P \leq 0.05$; Figure 5D]. CBDV also significantly contributed to the overall anticonvulsant effects of treatment on severity ($F_{1,110}$ = 22.711, $P \leq 0.001$; Figure 5A) and the incidence of tonic–clonic convulsions [$\chi^2(1) = 4.010$, $P \leq 0.01$; Figure 5C], although it had no significant effect on onset latency ($P > 0.1$; Figure 5D). The percentage of animals that remained convulsion-free [$\chi^2(6) = 1.564$, $P > 0.1$] was unaffected by treatment. No significant interactions between CBDV and VPA effects were observed (Supporting Information Tables S2, $P > 0.1$).

Co-administration of 200 mg·kg^{-1}CBDV and phenobarbital (10–40 mg·kg^{-1}) had significant anticonvulsant effects on the severity of pilocarpine-induced convulsions ($F_{7,108}$ = 19.352, $P \leq 0.001$; Figure 5E). When modelled with log-linear analysis, our data indicated that there was no effect of treatment on mortality (Figure 5F), whereas the percentage of animals that developed tonic–clonic convulsions was significantly decreased (Figure 5G). No effect of drug treatment

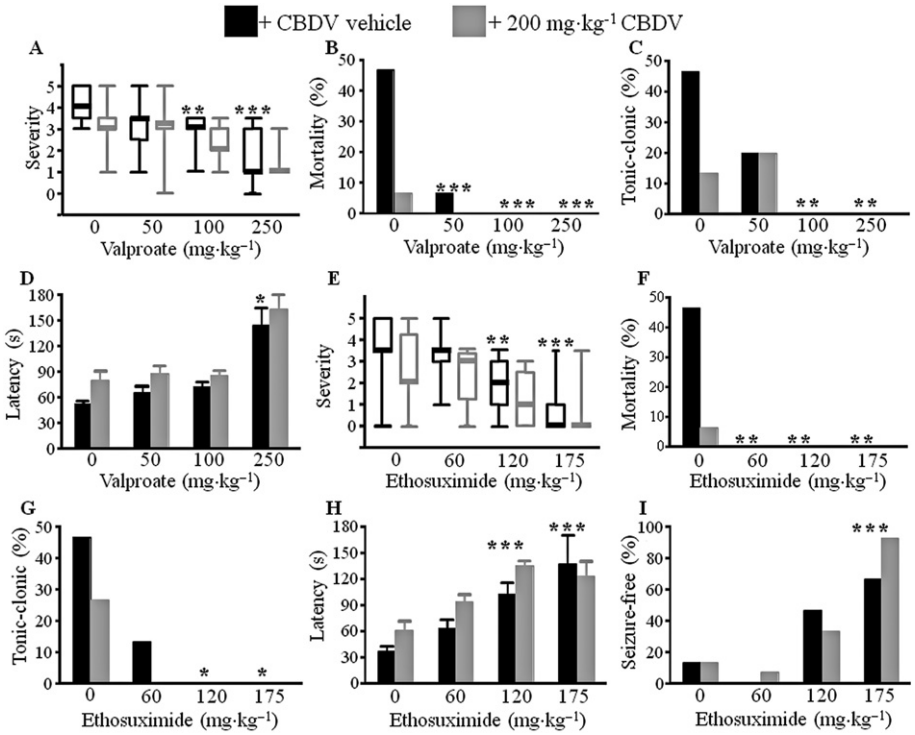

Figure 4

Effects of co-administration of CBDV and AEDs on PTZ-induced seizures in rats. The effects of CBDV co-administration with VPA (A–D) or ESM (E–I) on PTZ-induced seizures: severity (A and E), mortality (B and F), the incidence of tonic–clonic seizures (C and G), onset latency (D and H) and (for CBDV + ESM only) the percentage of animals that remained seizure-free. In (D and H), onset latency is presented as mean ± SEM. In (A and E), median severity is represented by a thick horizontal line, the 25th and 75th percentiles by the box and maxima and minima are represented by 'whiskers'. Significance of CBDV treatment is given in text. $n = 15$ in all cases. *$P \leq 0.05$, **$P \leq 0.01$ and ***$P \leq 0.001$ for AED effects.

was observed on seizure onset latency ($P > 0.1$; Figure 5H); however, when modelled with log-linear analysis, our data indicated that the percentage of animals that remained convulsion-free was significantly increased (Figure 5I). Phenobarbital significantly contributed to all anticonvulsant effects (Figure 5E–I; Supporting Information Table S2). CBDV significantly contributed to the overall decrease seen in severity ($F_{1,108} = 4.480$, $P \leq 0.05$), and the effects of CBDV and phenobarbital interacted significantly due to a convergence of the severity observed in the absence and presence of CBDV (Figure 5F, Supporting Information Table S2; $F_{3,108} = 3.105$, $P \leq 0.05$), no further significant interactions between the effects of CBDV and phenobarbital were observed ($P > 0.1$; Supporting Information Table S2).

Data from the co-administration experiments demonstrate that the AEDs strongly suppress PTZ-induced seizures and pilocarpine-induced convulsions in a dose-dependent manner (Figures 4 and 5). From several, but not all, of the parameters examined, 200 mg·kg^{-1} CBDV significantly contributed to the anticonvulsant effects observed in these experiments. To more precisely assess the effect of CBDV on AED actions in these studies, we performed pairwise comparisons at each dose of AED between groups that received CBDV vehicle and groups that received 200 mg·kg^{-1} CBDV; these analyses were only performed if two-way ANOVA or log-linear analysis results indicated an overall effect of CBDV upon a given parameter. Based on these analyses and Figure 5F–I, the effect of CBDV on the actions of phenobarbital in the pilocarpine model appears limited and is not significant. Similarly, the effect of CBDV on the actions of VPA in the PTZ model was limited (Figure 4A–D); the primary effect of CBDV is on delaying seizure onset, as 200 mg·kg^{-1} CBDV significantly improved the effect of 50 mg·kg^{-1} VPA ($P \leq 0.05$; Figure 4D) and showed a statistical trend towards the same effect with 100 mg·kg^{-1} VPA ($P < 0.1$). More notably, CBDV significantly improved the effect of 60 mg·kg^{-1} ESM on PTZ-induced seizure severity and onset latency ($P \leq 0.05$; Figure 4E and H) and also showed a statistical trend to improvement of the 120 mg·kg^{-1} ESM effect for both these measures ($P < 0.1$). Furthermore, when 200 mg·kg^{-1} CBDV was administered together with VPA before pilocarpine administration, it significantly improved the effects of VPA on severity (62.5 and 250 mg·kg^{-1}; $P \leq 0.05$), mortality (62.5 and 125 mg·kg^{-1}; $P \leq 0.05$) and the percentage of animals that experienced the most severe seizures (all doses, $P \leq 0.01$; Figure 5A–C).

Thus, CBDV is well-tolerated when co-administered with AEDs and does not interact antagonistically with any of the AEDs studied in either seizure model. Furthermore, CBDV has significant anticonvulsant effects when co-administered with ESM in the PTZ model and even greater effects when co-administered with VPA in the pilocarpine model, where beneficial effects were generally observed at low and medium AED doses. CBDV did not affect the effects of phenobarbital

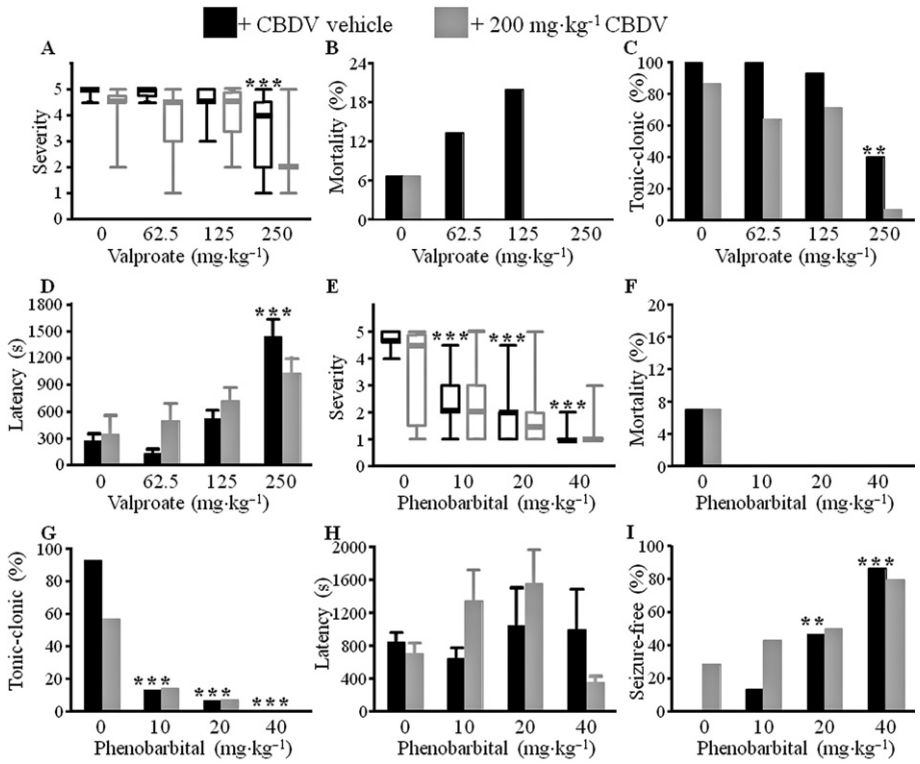

Figure 5

Effects of co-administration of CBDV and AEDs on pilocarpine-induced convulsions in rats. The effects of CBDV co-administration with VPA (A–D) or phenobarbital (E–I) on pilocarpine-induced convulsions: severity (A and E), mortality (B and F), the incidence of tonic–clonic convulsions (C and G), onset latency (D and H) and (for CBDV + phenobarbital only) the percentage of animals that remained seizure-free. In (D and H), onset latency is presented as mean ± SEM In (A and E), median severity is represented by a thick horizontal line, the 25th and 75th percentiles by the box and maxima and minima are represented by 'whiskers'. Significance of CBDV treatment is given in text. $n = 15$ in all cases. *$P \leq 0.05$, **$P \leq 0.01$ and ***$P \leq 0.001$ for AED effects.

in the pilocarpine model and had only very limited effects on the onset of seizures when co-administered with VPA before PTZ treatment.

CBDV motor side effect profile and anticonvulsant efficacy when administered p.o.

To further determine the suitability of CBDV as a clinical candidate, we assessed both its motor side effect profile and whether it could suppress seizures when administered p.o. before PTZ treatment. Many currently used AEDs have significant side effects at clinically effective doses, particularly on motor function (Schachter, 2007). Additionally, a prerequisite for human epilepsy treatment is that a drug is effective after oral administration.

We used two motor tasks to investigate the side effect profile of CBDV (50–200 mg·kg^{-1}): a static beam test to assess motor coordination (Stanley *et al.*, 2005; Roberts *et al.*, 2006) and a grip strength test to assess drug-induced muscle relaxation and functional neurotoxicity (Nevins *et al.*, 1993; Crofton *et al.*, 1996). CBDV had no significant effects on motor performance at any dose compared with vehicle treatment (Figure 6A–D). In the static beam assay, the pass rate [$\chi^2(3) = 4.053$; $P > 0.1$; Figure 6A] and mean distance travelled ($F_{3,79} = 1.335$; $P > 0.1$; data not shown) were both unaffected by CBDV. CBDV had no significant overall effect on the mean number of foot slips ($F_{3,79} = 0.858$; $P > 0.1$; Figure 6B), although we did note a non-significant increase in foot slips in animals treated with 200 mg·kg^{-1} CBDV (0.70 ± 0.25 slips, compared with 0.30 ± 0.11 slips after vehicle treatment). CBDV had no effect on grip strength ($F_{3,79} = 0.465$; $P > 0.1$, Figure 6C). To validate the tests' ability to detect AED-induced motor deficits, a second group of animals received VPA (125–350 mg·kg^{-1}) or saline vehicle. VPA significantly affected the percentage of animals that successfully completed the static beam test [$\chi^2(3) = 35.084$; $P \leq 0.001$; Figure 6A], with doses ≥250 mg·kg^{-1} significantly decreasing the pass rate ($P \leq 0.01$). Similarly, both the number of foot slips ($F_{3,78} = 9.140$; $P \leq 0.001$; Figure 6B) and the mean distance travelled ($F_{3,78} = 15.561$; $P \leq 0.001$; data not shown) were significantly, negatively and dose-dependently affected by treatment with ≥250 mg·kg^{-1} VPA ($P \leq 0.01$). VPA also significantly affected the grip strength of animals ($F_{3,79} = 3.175$; $P \leq 0.05$; Figure 6C), with a small, but significant decrease in mean strength induced by 350 mg·kg^{-1} VPA ($P \leq 0.05$).

Finally, we investigated the ability of 400 mg·kg^{-1} CBDV administered p.o. (see Supporting Information Appendix S1 for dose details) to suppress PTZ seizures (90 mg·kg^{-1});

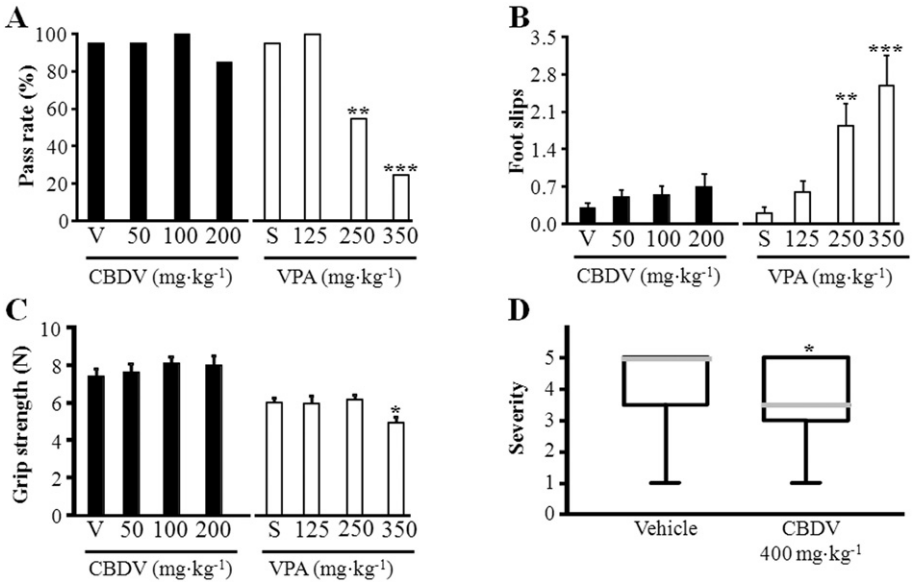

Figure 6

Effects of CBDV on performance in the static beam and forelimb grip strength assays in rat and as an orally administered anticonvulsant. (A and B): static beam performance; including the pass rate (A) and foot slips (B). (C) Performance in the grip strength assay. (A) Pass rate is represented as percentage; (B and C), represented as mean ± SEM n = 20 for static beam data, 10 for grip strength. (D) Effect of orally administered 400 mg·kg^{-1} CBDV on the severity of PTZ-induced seizures. (A–C): n = 20, (D): n = 15. *$P \leq 0.05$, **$P \leq 0.01$ and ***$P \leq 0.001$ respectively. (A–C): V = CBDV vehicle; S = VPA vehicle (saline).

400 mg·kg^{-1} CBDV significantly lowered the severity of PTZ-induced seizures (Figure 6D, $P \leq 0.05$) from 5 to 3.5. There were no significant effects of CBDV on seizure onset latency (vehicle 58.6 ± 3.7 s; CBDV 61.8 ± 5.2 s; $P > 0.1$), percentage mortality (vehicle 26.7%; CBDV 20%; $P > 0.1$) or development of tonic–clonic seizures (vehicle 53.3; CBDV 33.3; $P > 0.1$). Overall, we demonstrated that the anticonvulsant effects of CBDV in rat are due to genuine anticonvulsant properties and not motor suppression, and that CBDV is anticonvulsant when administered p.o. as well as i.p. in the PTZ model.

Discussion

This study demonstrates, for the first time, that CBDV has anticonvulsant properties, and, to date, is the only study that has investigated the effects of CBDV in whole animals. Our main finding is that CBDV suppresses seizures in four *in vivo* seizure models at doses ≥50 mg·kg^{-1}. CBDV also did not affect normal motor function and was well-tolerated when co-administered with AEDs. Moreover, CBDV suppressed epileptiform activity *in vitro*.

In vitro *effects of CBDV*

In both *in vitro* models of epileptiform activity, LFP duration and amplitude were significantly decreased by CBDV, with efficacy varying between hippocampal subregions and models. The CA3 region was most resistant to CBDV effects, potentially due to its role as the epileptiform focus (Perreault and Avoli, 1992; Hill *et al.*, 2010). It has also been reported that a smaller proportion of neurons in the CA1 contribute to burst activity than in the CA3 (Perreault and Avoli, 1992), potentially rendering the CA1 region more sensitive to the effects of anti-epileptiform drugs. CBDV effects on LFP frequency in the two models were opposite; CBDV increased Mg^{2+}-free-induced LFP frequency, but decreased 4-AP-induced LFP frequency. This may be due to a genuine, model-dependent CBDV effect on LFP frequency; however, the response of frequency in the Mg^{2+}-free model is in direct contrast to all other findings across both models, where varying degrees of anti-epileptiform effects were observed. In addition, we have observed that LFPs in the Mg^{2+}-free model exhibit greater variation in frequency than the 4-AP model; sporadic bursts of LFPs occur with periods of relative quiescence between them (see Hill *et al.*, 2010). Thus, while the frequency of LFPs in this Mg^{2+}-free model was corrected to allow for inherent increases, it may be that the unpredictability of LFP incidence limits the accuracy of this process. Overall, the magnitude of the effects of CBDV on LFP amplitude and duration are comparable with those observed with both CBD and clinically used AEDs (Sagratella, 1998; Hill *et al.*, 2010; Jones *et al.*, 2010).

In vivo *effects of CBDV and clinical implications*

We demonstrated that CBDV has significant anticonvulsant effects in four seizure models with different bases across two species. CBDV was effective in three models of generalized seizure – mES and audiogenic in mice and PTZ in rats. In particular, CBDV (200 mg·kg^{-1}) completely prevented tonic–clonic convulsions in the audiogenic seizure model and had robust effects in the mES model, in line with the reported

efficacy of VPA and other AEDs in these models (Gareri *et al.*, 2004; Luszczki *et al.*, 2011; 2012). Moreover, positive findings in the mES model – a primary screen for putative anticonvulsants (Loscher, 2011) – are predictive of clinical efficacy against generalized human seizures (Loscher, 2011). Audiogenic seizures, although providing limited predictive differentiation of future efficacy against human seizure types (Loscher, 2011), are also a useful model of generalized seizure (Pitkanen *et al.*, 2006). Attenuation of PTZ-induced seizures can be predictive of efficacy against absence seizures, as well as predicting effective suppression of generalized seizures in humans (Veliskova, 2006). Hence, CBDV should also be investigated in non-convulsive seizure models (e.g. WAG/Rjj rats; Coenen and Van Luijtelaar, 2003). Importantly, p.o. CBDV (400 mg·kg^{-1}) also suppressed PTZ-induced seizures, showing that CBDV can exert anticonvulsant effects when administered orally.

Systemic administration of pilocarpine induces *status epilepticus* with a temporal lobe focus that subsequently generalizes and is associated with motor convulsions (Curia *et al.*, 2008). Interestingly, the anticonvulsant effects of CBDV only became apparent when 200 mg·kg^{-1} CBDV and AEDs were co-administered. Thus, effects were observed only in higher-power experiments in which 60, as opposed to 15, animals received 200 mg·kg^{-1} CBDV. These effects were limited (see later), suggesting that CBDV is less effective in this model than in the others studied here. However, our statistical analyses revealed that the effects of CBDV in these experiments were independent of, and separate from, the actions of AED. Hence, it would be of interest to characterize the effects of CBDV on pilocarpine-induced *status epilepticus* using direct recordings of brain activity, for example via electroencephalographic or electrocorticographic recordings in this model as *status epilepticus* activity can persist in the absence of motor activity.

Many AEDs exert significant motor side effects (Schachter, 2007), which can limit patient quality of life. To address this and confirm that CBDV's anticonvulsant actions were due to direct actions on seizures and not motor suppression, we investigated the effects of CBDV on the performance of rats in the static beam and grip strength tasks. These tests assess balance, coordination, muscle relaxation and drug-induced functional neurotoxicity (Nevins *et al.*, 1993; Crofton *et al.*, 1996; Stanley *et al.*, 2005; Muller *et al.*, 2008). CBDV did not affect grip strength, and although the number of foot slips did increase after 200 mg·kg^{-1} CBDV treatment, this effect was not significant. Our tests were validated by the finding that, consistent with previous studies, VPA negatively affected all motor parameters (Roks *et al.*, 1999).

Our *in vivo* results showing that CBDV has comparatively strong anticonvulsant effects in a range of seizure models, indicate that CBDV has significant potential for the treatment of generalized, human seizures and should be further investigated against temporal lobe seizures. Furthermore, data from the motor function assays indicate that CBDV does not have significant adverse motor effects at anticonvulsant doses. In the future, it will be of great interest to investigate CBDV's properties in models of chronic epilepsy and hyperexcitability. The effect of chronic CBDV treatment on behaviour in healthy and epileptic animals is also worthy of investigation.

Co-administration studies

Clinical investigation of new anticonvulsants is typically performed using the candidate AED as an adjunctive treatment to the patient's current treatment regimen (French *et al.*, 2001). Therefore, we investigated the effects of CBDV (200 mg·kg^{-1}) when co-administered with clinically used anticonvulsants. The three anticonvulsants used were chosen based on their use as prescribed AEDs and, more pragmatically, reported efficacy in the seizure models used (Loscher *et al.*, 1991; Sofia *et al.*, 1993; Shantilal *et al.*, 1999; Lindekens *et al.*, 2000; Loscher, 2011). No negative interactions between CBDV and the AEDs were observed, indicating that CBDV is well-tolerated when co-administered with the three clinically used AEDs employed in these studies. The anticonvulsant effect of CBDV beyond that of these AEDs was variable, in our study. When administered with ESM before PTZ or VPA before pilocarpine, CBDV contributed significantly to the effects seen on severity (both cases), mortality (VPA in pilocarpine only), latency (ESM only) and the incidence of tonic–clonic convulsions (VPA in pilocarpine only). The majority of the significant facilitatory effects of CBDV were seen at the lower two doses; this could be due to the greater potential for anticonvulsant actions when the AED is not producing a maximal effect itself. However, 200 mg·kg^{-1} CBDV appeared to have little effect on pilocarpine-induced convulsions when administered with phenobarbital at any dose, although it should be noted that all doses of phenobarbital strongly suppressed seizure activity, probably limiting CBDV's effect. CBDV had limited effects on PTZ-induced seizures when co-administered with VPA. Thus, CBDV had AED-dependent effects in these experiments, producing notable improvements over AED treatment alone in two of four experiments. Based on these data, we postulate that CBDV is well-tolerated when co-administered with three AEDs used in the clinic for a variety of epileptic syndromes, but that further investigation of its anticonvulsant properties in combination with other drugs is required, for example, using isobolographic experimental design and analysis (e.g. Luszczki *et al.*, 2010).

Anticonvulsant mechanisms of CBDV

This is the first investigation of CBDV effects in any *in vivo* model or system; *in vitro* information on CBDV pharmacological properties, while growing, is limited (Scutt and Williamson, 2007; De Petrocellis *et al.*, 2011a,b) and remains of unknown *in vivo* or clinical relevance. For example, reported effects of CBDV at recombinant TRP channels are as yet unconfirmed in native tissue and it is unknown how such TRP-based mechanisms of action could affect excitability in epileptogenic areas. While TRPV1 expression in brain areas including the hippocampus remain controversial (Mezey *et al.*, 2000; Cavanaugh *et al.*, 2011), the functional expression of other TRP subtypes in relevant parts of the brain has yet to be confirmed (Crawford *et al.*, 2009; Hirata and Oku, 2010). CBDV has also been reported to inhibit diacylglycerol lipase (DAGL) α (De Petrocellis *et al.*, 2011a), the enzyme responsible for the production of the endocannabinoid 2-arachidonoylglycerol (2-AG; Stella *et al.*, 1997). The effect of inhibiting 2-AG production is likely to be complex. The initial effect would be to decrease 2-AG levels and subsequent activation of CB_1 cannabinoid receptors. However, the overall effect of this on seizure activity would depend on propor-

tional CB_1 cannabinoid receptor expression and localization on different presynapses (i.e. excitatory or inhibitory), and the contribution of inhibitory GABAergic circuits in brain areas crucial to epileptogenesis, as a decrease in 2-AG would result in less suppression of both excitatory and inhibitory synapses. Furthermore, over longer time courses, it has been reported that CB_1 cannabinoid receptor levels can be affected by changes in agonist levels, that is higher levels of CB_1 cannabinoid receptor agonists can increase internalization of the receptor (Coutts *et al.*, 2001). Thus, reduced 2-AG levels could cause increased the number of CB_1 cannabinoid receptors at the membrane. In addition, in this study the effects of CBDV were only investigated on acute seizures and CB_1 cannabinoid receptor expression changes during both animal models (e.g. pilocarpine-induced spontaneous recurrent seizures as a model of temporal lobe epilepsy) of chronic epilepsy and in human epilepsy (Magloczky *et al.*, 2010; Karlocai *et al.*, 2011), which could affect the consequences of changes in endocannabinoid levels upon seizure activity. Δ^9-THC has been reported to have a direct anticonvulsant action via CB_1 cannabinoid receptor agonism (Wallace *et al.*, 2001). However, the effects of CBDV on CB_1 cannabinoid receptors have not been characterized. Furthermore, 200 $mg \cdot kg^{-1}$ CBDV had no significant effects in the motor function assays used here, whereas CB_1 cannabinoid receptor agonists produce significant motor deficits (Carlini *et al.*, 1974), which suggests that CBDV does not act via CB_1 cannabinoid receptor agonism.

CBDV is the propyl analogue of CBD and a qualitative comparison of the effects of CBD and CBDV on PTZ-induced seizures showed that both compounds improve mortality and severity. However, CBD produced these effects at 100 $mg \cdot kg^{-1}$, a dose at which CBDV did not affect severity. CBD did not appear to affect onset latency ($\leq$100 $mg \cdot kg^{-1}$), whereas CBDV delayed seizure onset in a dose-dependent manner that reached significance at 200 $mg \cdot kg^{-1}$. The comparison between CBD and CBDV in the pilocarpine model is less simple as CBDV at 200 $mg \cdot kg^{-1}$ had wider-ranging anticonvulsant effects in our co-administration experiments (on severity, mortality and latency as well as the proportion of animals that developed tonic–clonic convulsions), but was not effective in initial experiments at any dose, whereas low-dose CBD affected tonic–clonic convulsions, but no other measures. Hence, it would be of interest to perform a direct experimental comparison both of efficacy and how similarly CBD and CBDV affect seizures. Although assumptions of pharmacological similarity between plant cannabinoids on the basis of structural homology should be made with caution (e.g. the opposing effects of Δ^9-THC and Δ^9-THCV on CB_1 cannabinoid receptors), CBD is anticonvulsant in animals and humans, and more is known about CBD's pharmacological properties, if not its specific anticonvulsant mechanism(s) of action. CBD has a wide range of known pharmacological targets, which are unlikely to include CB_1 cannabinoid receptors, that could underlie its anticonvulsant effects (Hill *et al.*, 2012). These include inhibition of T-type Ca^{2+} channels (Ross *et al.*, 2008), inhibition of GPR55 in some tissues/preparations (Ryberg *et al.*, 2007), modulation of mitochondrial calcium handling in neurons (Ryan *et al.*, 2009) and increased activity of inhibitory non-cannabinoid GPCRs including $5\text{-}HT_{1A}$ (direct agonism; Russo *et al.*, 2005) and adenosine A_1 (via effects on adenosine uptake; Carrier *et al.*, 2006). Thus, if CBDV shares some or all of CBD's pharmacological targets, it is possible that CBDV also acts via multiple mechanisms to produce its overall anticonvulsant effect, as opposed to exerting a high-efficacy action at a single target. However, there is no *a priori* reason to assume a common target and there is clearly some divergence between the properties of CBD and CBDV, for example CBD, but not CBDV, inhibits FAAH (De Petrocellis *et al.*, 2011a).

In conclusion, our most important finding is that CBDV possesses strong anticonvulsant properties in a range of *in vivo* seizure models that parallel a variety of human seizure types and pathologies; anticonvulsant effects were also seen after oral, as well as i.p., administration. As with many clinically used AEDs, further work is required to determine the anticonvulsant mechanism of CBDV, but the significant anticonvulsant effects and favourable motor side effect profile demonstrated in this study identify CBDV as a potential standalone AED or as a clinically useful adjunctive treatment alongside other AEDs.

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Conflict of interest

The work reported was funded by grants to BJW, CMW & GJS from GW Pharmaceuticals and Otsuka Pharmaceuticals. BJW, AJH, NAJ, CMW & GJS were responsible for experimental design. YY and TF are employees of Otsuka Pharmaceuticals and hold stocks in this company. MD and CGS are GW Pharmaceuticals employees, and CGS is a stockholder.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Methods.
Tables S1 and S2 For each seizure parameter that was affected by CBDV + AED treatment, the analysis of the individual AED effect is given (either as ANOVA or Chi-squared). The directions of significant effects are also given by an upward or downward arrow (irrespective of the parameter, all significant AED effects described are anticonvulsant). Additionally, the doses at which AEDs were significantly anticonvulsant are indicated with *post hoc* p values given after. Finally, analyses of interactions between CBDV and AED effects are given.