Epilepsia, 51(8):1522–1532, 2010
doi: 10.1111/j.1528-1167.2010.02523.x

FULL-LENGTH ORIGINAL RESEARCH

Δ^9-Tetrahydrocannabivarin suppresses in vitro epileptiform and in vivo seizure activity in adult rats

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Summary

Purpose: We assessed the anticonvulsant potential of the phytocannabinoid Δ^9-tetrahydrocannabivarin (Δ^9-THCV) by investigating its effects in an in vitro piriform cortex (PC) brain slice model of epileptiform activity, on cannabinoid CB1 receptor radioligand-binding assays and in a generalized seizure model in rats.

Methods: Δ^9-THCV was applied before (10 μM Δ^9-THCV) or during (10–50 μM Δ^9-THCV) epileptiform activity induced by Mg^{2+}-free extracellular media in adult rat PC slices and measured using multielectrode array (MEA) extracellular electrophysiologic techniques. The actions of Δ^9-THCV on CB1 receptors were examined using [^{3}H]SR141716A competition binding and [^{35}S]GTPγS assays in rat cortical membranes. Effects of Δ^9-THCV (0.025–2.5 mg/kg) on pentylenetetrazole (PTZ)–induced seizures in adult rats were also assessed.

Results: After induction of stable spontaneous epileptiform activity, acute Δ^9-THCV application (≥20 μM) significantly reduced burst complex incidence and the amplitude and frequency of paroxysmal depolarizing shifts (PDSs). Furthermore, slices pretreated with 10 μM Δ^9-THCV prior to induction of epileptiform activity exhibited significantly reduced burst complex incidence and PDS peak amplitude. In radioligand-binding experiments, Δ^9-THCV acted as a CB1 receptor ligand, displacing 0.5 nM [^{3}H]SR141716A with a Ki∼290 nM, but exerted no agonist stimulation of [^{35}S]GTPγS binding. In PTZ-induced seizures in vivo, 0.25 mg/kg Δ^9-THCV significantly reduced seizure incidence.

Discussion: These data demonstrate that Δ^9-THCV exerts antiepileptiform and anticonvulsant properties, actions that are consistent with a CB1 receptor–mediated mechanism and suggest possible therapeutic application in the treatment of pathophysiologic hyperexcitability states.

KEY WORDS: Epilepsy, Anticonvulsant, Cannabinoid, Pentylenetetrazole, Piriform cortex.

Epilepsy is a chronic and debilitating neurologic disorder affecting approximately 1% of the global population. *Cannabis sativa* has historically been used to alleviate the symptoms of epilepsy. However, cannabis has been ascribed both pro- (Brust et al., 1992) and anticonvulsant (Alger, 2004; Gross et al., 2004) properties, while the literature concerning cannabis and epilepsy is somewhat limited in scope and frequently contradictory (Lutz, 2004). It has been postulated that differing relative proportions of the distinct constituent cannabinoids, which currently number ∼60, can alter cannabis effects in patients with epilepsy (Ashton, 2001). Therefore, the composition of differing cannabis strains and epilepsy's complex etiology may explain the markedly different, even opposing, effects of cannabis upon the induction, maintenance, and propagation of hyperexcitability states.

The principal site of action of the major psychoactive component of cannabis, Δ^9-tetrahydrocannabinol (Δ^9-THC), in the central nervous system (CNS) is CB1 receptors (Compton et al., 1996), which are also activated by endocannabinoids [principally anandamide (AEA) and 2-arachidonoylglycerol (2-AG)]; these interactions have been implicated in numerous physiologic and pathophysiologic roles (Piazza et al., 2007; Di Marzo, 2008). Principal among these interactions with regard to the balance and control of excitability in the CNS is the retrograde attenuation of both excitatory and inhibitory vesicular neurotransmitter release at presynaptic terminals (Alger et al., 1996). Therefore, Δ^9-THC, endocannabinoids, and exogenous CB1 receptor ligands may exert complex effects on neuronal excitability and synchronization (Mason & Cheer, 2009) in specific brain regions and across the CNS, dependent on receptor localization to specific neuronal synapses.

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Accepted January 7, 2010; Early View publication February 26, 2010.

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Δ^9-tetrahydrocannabivarin (THCV) is a relatively abundant nonpsychoactive phytocannabinoid present in *Cannabis sativa* (Gill et al., 1970), and has recently been shown to be a CB1-receptor antagonist. Therefore, Δ^9-THCV displaced radiolabeled CB1 agonists in mouse whole brain membranes (Thomas et al., 2005), and antagonized CB1-specific GTPγS binding in mouse whole brain, cerebellar, and piriform cortex (PC) membranes (Thomas et al., 2005; Dennis et al., 2008). We have previously shown that Δ^9-THCV reduced Purkinje cell firing via an increase in inhibitory neurotransmission at interneuron–Purkinje cell synapses in mouse acute parasagittal cerebellar brain slices, most likely by reducing CB1 receptor–mediated, endocannabinoid-induced inhibition of γ-aminobutyric acid (GABA) release (Ma et al., 2008). Interestingly, Δ^9-THCV was shown to modulate GABA release onto Purkinje cells at a network level, as it did not affect Purkinje cell spike firing following GABA-receptor blockade.

In the present study, we have used extracellular multielectrode array (MEA) recording methods to investigate the effects of Δ^9-THCV upon spontaneous epileptiform bursting in the PC induced by omitting Mg^{2+} ions from the extracellular solution (Whalley et al., 2009). The PC is part of the limbic system and is responsible for the assimilation and integration of odor responses into memory (Hasselmo et al., 1990; Barkai & Hasselmo, 1997), in addition to playing an important role in the genesis and spread of temporal lobe epilepsy with complex partial seizures (Loscher & Ebert, 1996; Ebert et al., 2000). Therefore, the transverse PC slice preparation provides a relevant and useful model for examining candidate anticonvulsant drug effects in vitro (Whalley et al., 2005, 2009). Overall, we show that Δ^9-THCV exerted significant antiepileptiform effects, reducing burst complex incidence and paroxysmal depolarizing shift (PDS) amplitude and frequency following acute application and reducing burst complex incidence and PDS peak amplitude following pretreatment. In vitro binding assays suggest a relatively high affinity interaction with CB1 receptors but a lack of agonist action. In pentylenetetrazole (PTZ) –induced seizures in vivo, 0.25 mg/kg, Δ^9-THCV reduced seizure incidence. Therefore, Δ^9-THCV possesses antiepileptiform and anticonvulsant properties, suggesting potential usefulness in attenuation of pathophysiologic hyperexcitability states.

Methods

In vitro electrophysiology

Tissue preparation and solutions

All experiments were carried out in accordance with Home Office regulations (Animals (Scientific Procedures) Act 1986). Acute transverse PC brain slices (~450 μm thick) were prepared from male and female adult outbred rats (p > 40; Berkshire) as described previously (Whalley et al., 2005) in an artificial cerebrospinal fluid (aCSF) solution of composition (in mM): NaCl 118; KCl 3; $NaHCO_3$ 25; $MgCl_2.6H_2O$ 1; $CaCl_2$ 2.5, and D-glucose 11 at 25°C (Fisher Scientific, Loughborough, United Kingdom). All solutions were continuously carboxygenated with 95% O_2:5% CO_2; pH 7.4. Spontaneous epileptiform activity was induced by exchange of the standard aCSF perfusion media for aCSF with $MgSO_4.6H_2O$ removed (hereafter "Mg^{2+}-free aCSF"; Whalley et al., 2009). Omission of Mg^{2+} removes the physical block of the *N*-methyl-D-aspartate (NMDA) receptor by this ion at resting membrane potentials, permitting NMDA-receptor activation without concomitant depolarization.

MEA recordings

Substrate-integrated MEAs (Multi Channel Systems, Reutlingen, Germany) (Egert et al., 2002a,b) were used to electrophysiologically record spontaneous neuronal activity. MEAs comprised 60 electrodes of 30-μm diameter, in an "~8 × 8" arrangement with 200-μm spacing between electrodes. MEAs were cleaned before each recording using sequential treatments of 5% w/v Terg-A-Zyme (Cole-Palmer, Hanwell, United Kingdom) in dH_2O, methanol, and, finally, distilled water before air drying. Slices were adhered to the cleaned MEA surface using an applied (~4 μl) and evaporated cellulose nitrate solution in methanol (0.24% w/v Protran Nitrocellulose Transfer Membranes; Schleicher & Schuell Bioscience Inc., Keene, NH, U.S.A.). Slice position upon the MEA was determined by observation at magnification ×4 with a Nikon TS-51 (Nikon, Tokyo, Japan) inverted microscope, and imaged via a Mikro-Okular camera (Meade Instruments Corp., Irvine, CA, U.S.A.) (Fig. 1A). Once adhered, slices were maintained at 25°C, continuously superfused (2–4 ml/min) with carboxygenated aCSF, and allowed to stabilize for at least 10 min prior to any recordings. Δ^9-THCV (GW Pharmaceuticals, Porton Down, United Kingdom) was stored as a stock solution in dimethylsufoxide (DMSO). Full details of signal processing, pharmacology protocols, and data analyses may be found in Data S1.

Receptor and GTPγS binding assays

Cortical tissue was dissected from the brains of adult (p > 21) male and female Wistar Kyoto rats. Cortical membranes were prepared, and radioligand saturation and competition binding assays using the selective CB1 receptor antagonist [^{3}H]SR141716A and [^{35}S]GTPγS binding assays were performed, both as described previously in (Jones et al. 2009). See Data S1 for full details.

Chemically induced in vivo seizure model

Chemically induced seizures

Eighty milligrams per kilogram PTZ (Sigma, Poole, United Kingdom) was used to induce seizures in 64 adult (>P21, 70–110 g) male Wistar rats. PTZ is a GABA-recep-

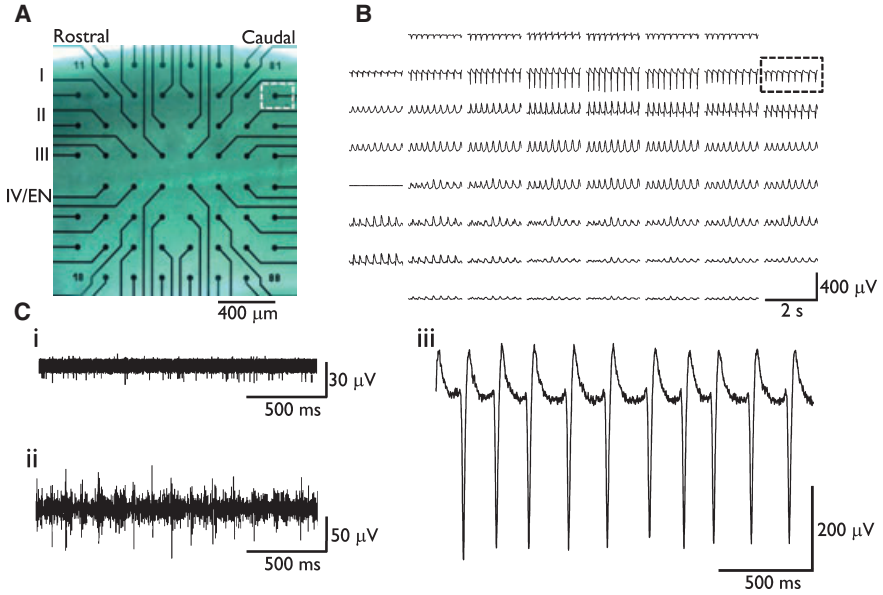

Figure 1.
Mg^{2+}-free artificial cerebrospinal fluid (aCSF) –induced epileptiform bursting in a piriform cortex (PC) slice. (**A**) An acute PC slice mounted on a multielectrode array (MEA). Numerals refer to PC layers, dotted box [also shown in (**B**)] highlights the electrode at which epileptiform activity first manifested and was used to produce representative single electrode data. (**B**) Representative plot displaying epileptiform activity across all 60 channels on the MEA. (**C**) High-pass filtered (100 Hz) data showing spike activity in (**i**) control aCSF and (**ii**) in Mg^{2+}-free aCSF during epileptiform activity. (**iii**) Expanded representative trace of a single burst from electrode marked in **A** and **B** (dotted boxes). All illustrated data are taken from the same slice.

tor antagonist, the application of which leads to neuronal disinhibition and seizures analogous to generalized human seizures (Olsen, 1981; Zhao & Holmes, 2006). In the days prior to seizure induction, animals were habituated to handling, experimental procedure, and environments. Animals were placed in a 6 L Perspex tank with lid and then injected intraperitoneally (i.p.) with Δ^9-THCV (0.025, 0.25, or 2.5 mg/kg). The Δ^9-THCV vehicle was a 1:1:18 solution of ethanol:Cremophor (Sigma, Poole, United Kingdom):0.9% w/v NaCl. Δ^9-THCV is known to penetrate the blood–brain barrier such that 30 mg/kg delivered intraperitoneally in rats provides C_{max} = 1.6 μg/g at T_{max} = 30 min and, at the same dosage, no major toxicity, genotoxicity, or mutagenicity was reported (GW Pharmaceuticals Ltd., Norwich, United Kingdom; Study Report UNA-REP-05a/b). A group of animals that received volume-matched doses of vehicle alone served as a negative control. After 30 min Δ^9-THCV or vehicle administration, animals were injected with PTZ, 80 mg/kg, i.p. to induce seizures. A video observation system (Farrimond et al., 2009) was used to monitor the behavior of ≤5 animals simultaneously from Δ^9-THCV/vehicle administration until 30 min after seizure induction.

Seizure analysis

Videos of PTZ-induced seizures were scored off line with a standard seizure severity scale (Pohl & Mares, 1987), using The Observer Video-Pro software (Noldus, Wageningen, The Netherlands). The seizure scoring scale was as follows: 0, no change in behavior; 0.5, abnormal behavior (sniffing, excessive washing, orientation); 1, isolated myoclonic jerks; 2, atypical clonic seizure; 3, fully developed bilateral forelimb clonus; 3.5, forelimb clonus with tonic component and body twist; 4, tonic–clonic seizure with suppressed tonic phase; 5, fully developed tonic–clonic seizure. Specific markers of seizure behavior and development were assessed and compared between vehicle control and Δ^9-THCV groups. For each animal the latency (in seconds) from PTZ administration to first sign of seizure (typically a score of [1]; myoclonic jerk), myoclonic seizure ([3]), and full tonic-clonic seizure ([5]) was recorded. In addition, the duration of seizure, the percentage of animals that experienced no seizure signs, and the median severity (the median of maximum severity for animals in a group that experienced seizures) were calculated for each experimental group.

In vivo data presentation and statistics

For seizure duration and latency values, group means ± standard error of the mean (SEM) are presented. The median value for seizure severity and percentage values for seizure-free animals and mortality are also presented. Significant differences for latency and seizure duration and median severity were assessed using analysis of variance

(ANOVA) with post hoc Tukey tests. Differences in percentage mortality and seizure incidence were assessed by a nonparametric binomial test. In all cases $p \leq 0.05$ was considered to be significant.

Results

Mg^{2+}-free aCSF-induced epileptiform bursting in vitro

Application of Mg^{2+}-free aCSF to MEA-adhered PC slices (Fig. 1A) readily induced epileptiform activity (Fig. 1B,Ciii). Epileptiform activity was consistently (n = 37/39 slices) characterized by the spontaneous appearance of local field potentials (LFPs) representative of paroxysmal depolarizing shifts (PDSs) that initiated in PC layer II (dotted boxes in Fig. 1A,B) and spreading in a caudorostral direction across the slice preparation, parallel to the pial surface (13.2 ± 0.5 μm/ms; n = 7 slices). Interestingly, the direction of burst spread was opposite to the dominant direction of information flow within the PC (Haberly, 1985). PC slices in control aCSF did not exhibit any spontaneous LFP activity (n = 39/39 slices), although discrete unit or multiunit spike events were evident (Fig. 1Ci), and such events had a frequency of 32 ± 4 Hz (in n = 5 slices analyzed). Mg^{2+}-free aCSF treatment significantly increased the observed spike firing rate by approximately 6-fold from 32 ± 18 to 202 ± 22 Hz; (Fig. 1Ci,ii; $p < 0.001$, n = 5), consistent with the expected increase in neuronal activity accompanying epileptiform states.

Effect of acute Δ^9-THCV application

Δ^9-THCV (5–50 μM) was applied cumulatively to PC slices in which epileptiform activity had been established with Mg^{2+}-free aCSF. Δ^9-THCV (20–50 μM) significantly decreased burst incidence in a concentration-dependent manner, with a reduction from 2.17 ± 0.3 Hz (Mg^{2+}-free aCSF as a control) to 1.05 ± 0.4 Hz at 50 μM Δ^9-THCV (Fig. 2Ai; $p < 0.01$, n = 5). PDS amplitude was also significantly decreased by Δ^9-THCV (20–50 μM) in a concentration-dependent manner, with the greatest effect observed at 50 μM Δ^9-THCV (a reduction to 60 ± 4% of Mg^{2+}-free aCSF control value; Fig. 2Aii; $p < 0.01$, n = 5). Finally, Δ^9-THCV (20–50 μM) reduced PDS frequency in a concentration-dependent fashion, with the greatest effect observed at 50 μM Δ^9-THCV (a reduction to 38 ± 12% of Mg^{2+}-free aCSF control value; Fig. 2Aiii; $p < 0.01$, n = 5). Representative control burst recordings in Mg^{2+}-free aCSF (Fig. 2Bi) and in the presence of 40 μM Δ^9-THCV (Fig. 2Bii) illustrate changes in burst characteristics induced by Δ^9-THCV application. Figure 2Biii shows a control single burst in Mg^{2+}-free aCSF and in the presence of 40 μM Δ^9-THCV superimposed to illustrate Δ^9-THCV-induced reductions in PDS amplitude and frequency. As described in full in Data S1, no

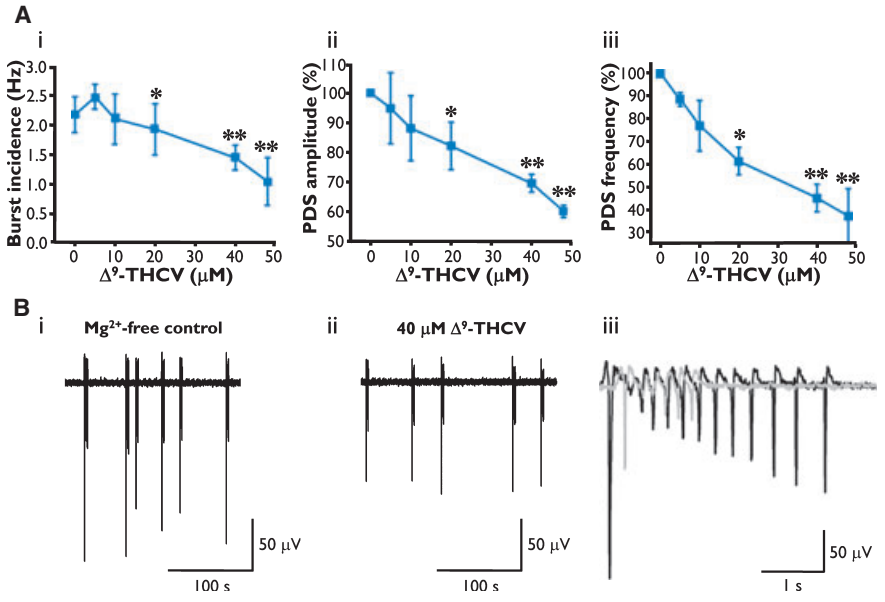

Figure 2.
Effects of acute treatment with Δ^9-tetrahydrocannabivarin (Δ^9–THCV) on epileptiform bursts. (**A**) Mean data showing the effects of Δ^9-THCV (5–50 μM) on control responses (**i**) burst incidence, (**ii**) paroxysmal depolarizing shift (PDS) amplitude as a percentage of control, (**iii**) PDS frequency as a percentage of control. (**B**) Representative PDS traces in (**i**) Mg^{2+}-free control, (**ii**) during 40 μM Δ^9-THCV application in Mg^{2+}-free artificial cerebrospinal fluid (aCSF), and (**iii**) expanded representative traces of bursts in the absence (black) and presence (gray) of 40 μM Δ^9-THCV. In **A**, data points are presented as mean values ± standard error of the mean (SEM) *$p \leq 0.05$; **$p \leq 0.01$. All experiments n = 5.

significant changes were seen in the preceding parameters in control experiments of a similar length (3.5 h) during which no Δ^9-THCV was added.

The effect of Δ^9-THCV upon the propagation of epileptiform activity within the PC slice preparation was further investigated using frames captured from contour plot animations of PDS amplitude changes (see Data S1). Contour plots enabled spatiotemporal visualization of PDS activity across the "8 × 8" MEA configuration (Fig. 3; c.f. Fig. 1B). Figure 3A shows discrete animation frames of spontaneous epileptiform activity in Mg^{2+}-free aCSF alone at quiescent (i.e., during inactivity, consistent with interictal periods; Fig. 3Ai), peak sink (Fig. 3Aii), and peak source (Fig. 3Aiii) states. Figure 3B shows the same states in the presence of 40 μM Δ^9-THCV and illustrates that PDS amplitude is clearly attenuated across the PC slice. In addition, Δ^9-THCV significantly decreased caudorostral epileptiform burst propagation speed from 13.2 ± 0.5 μm/ms in Mg^{2+}-free aCSF to 11.2 ± 0.2 μm/ms (40 μM Δ^9-THCV; $p < 0.01$, $n = 5$; Fig. 3C). These data suggest that Δ^9-THCV acts to significantly decrease the size, frequency, and incidence of epileptiform activity in the PC, as well as inhibiting its propagation across the PC.

Effects of pretreatment with Δ^9-THCV

We subsequently examined the effects of pretreating PC slices for 20 min with 10 μM Δ^9-THCV (a concentration that did not induce significant antiepileptiform effects in acutely treated slices; see Fig. 2A). Pre-treatment with 10 μM Δ^9-THCV prior to application of Mg^{2+}-free aCSF (Fig. 4Ciii,iv) significantly decreased both burst incidence (Fig. 4A; 2.17 ± 0.3 Hz Mg^{2+}-free aCSF only vs. 0.65 ± 0.21 Hz Mg^{2+}-free aCSF plus 10 μM Δ^9-THCV pre-treatment; $p < 0.01$, $n = 5$) and PDS amplitude (Fig. 4B; 197 ± 26 μV Mg^{2+}-free aCSF, 44 ± 11 μV Mg^{2+}-free aCSF plus 10 μM Δ^9-THCV pre-treatment; $p < 0.01$, $n = 5$). Notably, acute application of 10 μM Δ^9-THCV following the induction of epileptiform activity (Fig. 4Cv,vi) had no significant effect upon epileptiform burst incidence (2.17 ± 0.3 Hz Mg^{2+}-free aCSF only vs. 2.10 ± 0.42 Hz Mg^{2+}-free aCSF plus acutely applied 10 μM Δ^9-THCV; $p > 0.05$, $n = 5$) or PDS amplitude (Mg^{2+}-free aCSF plus acutely applied 10 μM Δ^9-THCV was 88 ± 11% of the value in Mg^{2+}-free aCSF alone; $p > 0.05$, $n = 5$) as illustrated in Fig. 2.

Data from the same series of experiments were also high-pass filtered to reveal unit and multiunit spiking activity between epileptiform events (see Fig. 1C). The induction of epileptiform activity by media exchange to Mg^{2+}free aCSF produced a significant decrease in mean interspike interval (ISI) (5.8 ± 3.7 ms; $n = 5$) when compared to control (27.5 ± 5.4 ms; $n = 5$, $p < 0.01$, Fig. 4D) consistent with increased levels of neuronal activity in epileptiform states. Acute application of 10 μM Δ^9-THCV after induction of epileptiform bursting did not further alter ISI (4.9 ± 1.9 ms; $p > 0.05$, $n = 5$), consistent with the absence of significant effects of acute 10 μM Δ^9-THCV upon previously described measures (Fig. 2).

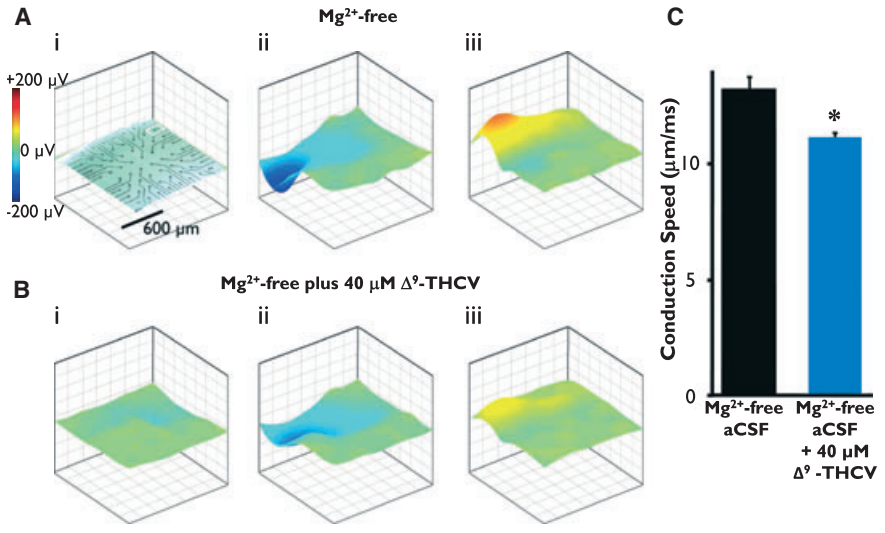

Figure 3.
Effects of Δ^9-THCV on Mg^{2+}-free artificial cerebrospinal fluid (aCSF)–induced burst propagation and spread. Contour plots of electrical activity induced by Mg^{2+}free aCSF of a representative piriform cortex (PC) slice on a multielectrode array (MEA) (**i**) between bursts, and (**ii**) at peak sink and (**iii**) peak source in the absence (**A**) and presence (**B**) of 40 μM Δ^9-THCV. Ai shows a PC slice with MEA electrodes aligned to a contour plot for illustrative purposes. (**C**) Effect of 40 μM Δ^9-THCV on propagation speed of epileptiform activity across the slice; data are presented as mean values ± SEM *$p \leq 0.05$, $n = 5$.

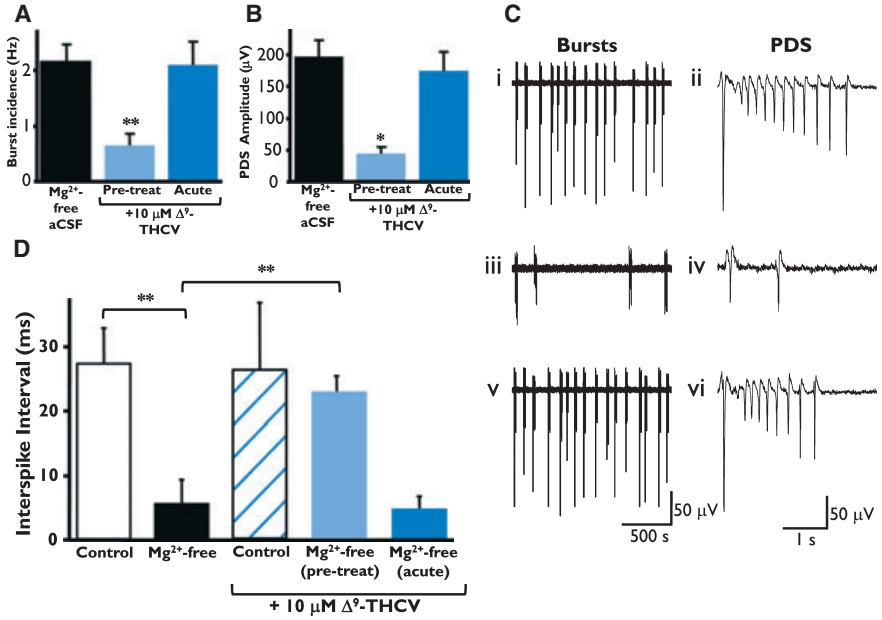

Figure 4.

Effects of Δ^9-THCV pre-treatment on 10 μM Δ^9-THCV effects on epileptiform bursts. Effect of 10 μM Δ^9-THCV in Mg^{2+}free aCSF applied acutely or following pretreatment with 10 μM Δ^9-THCV on (**A**) burst incidence (Hz) and (**B**) PDS amplitude (μV), n = 5 for both measures. (**C**) representative traces of burst complexes and single PDS recorded in Mg^{2+}-free aCSF (**i, ii**); Mg^{2+}-free aCSF with 10 μM Δ^9-tetrahydrocannabivarin (Δ^9-THCV) pretreatment (**iii, iv**) and Mg^{2+}-free aCSF with acute with 10 μM Δ^9-THCV (**v, vi**). **D**: Effects of Δ^9-THCV pretreatment on 10 μM Δ^9-THCV effects on interspike interval. Mean interspike intervals under different conditions; data are presented as mean values ± standard error of the mean (SEM) **p ≤ 0.01, all experiments n = 5.

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Moreover, pretreatment with 10 μM Δ^9-THCV in control aCSF revealed an effect upon ISI (23.0 ± 2.5 ms, n = 5, Fig. 4D) that was significantly longer than in Mg^{2+}-free aCSF alone ($p < 0.01$).

Effects Δ^9-THCV in receptor binding assays

In saturation binding assays, the CB1 receptor antagonist [^{3}H]SR141716A bound to rat cortical membranes according to a one-binding site model with B_{max}= 0.76 ± 0.07 pM/mg and K_d of 0.53 ± 0.01 nM (n = 3; Jones et al., 2010). Competition binding assays were performed for Δ^9-THCV against 0.5 nM [^{3}H]SR141716A, and effects were compared with those of the CB receptor agonist WIN55,212-2 and the CB1 receptor antagonist AM251 (Fig. 5A). AM251 displacement of [^{3}H]SR141716A binding was best fitted to a single high-affinity site (K_i = 190 ± 56 pM; Hill slope of −1.08 ± 0.13, n = 4; Jones et al., 2010). By contrast, WIN55,212-2 displacement was best fitted to a two binding–site model with high (K_i = 7.03 ± 4.1 nM; $\%R_h$ = 27.4 ± 5.0%; n = 4) and low (K_i = 904 ± 155 nM; n = 4) affinity sites; the Hill slopes for neither site matched unity (Jones et al., 2010). Δ^9-THCV displacement of [^{3}H]SR141716A occurred with lower affinity than AM251 (K_i = 286 ± 43 nM; n = 4), was best fitted by a one binding–site model and yielded a Hill slope of −0.81 ± 0.04.

We have previously shown that Δ^9-THCV reduced [^{35}S]GTPγS binding in mouse PC membranes (Dennis et al., 2008); we next investigated the effects of Δ^9-THCV on [^{35}S]GTPγS binding using the same membrane preparation used in the saturation and competition binding assays described earlier. Δ^9-THCV effects were compared with those of WIN55,212-2 and AM251 (Fig. 5B). WIN55,212-2 had a clear agonist effect causing an increase in the percentage stimulation of [^{35}S]GTPγS, whereas AM251 caused a moderate depression of [^{35}S]GTPγS binding (see Jones et al., 2010). Δ^9-THCV (10 pM – 10 μM) had no effect on [^{35}S]GTPγS binding; 100 μM Δ^9-THCV decreased [^{35}S]GTPγS binding (−76.7 ± 15.9%, n = 3), in agreement with our previous data for Δ^9-THCV in mouse membranes (Dennis et al., 2008).

Taken together, these data suggest that Δ^9-THCV acts as a CB1 ligand in the mammalian cortex and are consistent with previous reports of Δ^9-THCV effects in other species and brain areas (Thomas et al., 2005; Dennis et al., 2008).

Effects of Δ^9-THCV on PTZ-induced seizures

PTZ (80 mg/kg) induced seizures of varying severities in animals from all four experimental groups (vehicle, 0.025, 0.25, and 2.5 mg/kg Δ^9-THCV; n = 16 per group). PTZ-induced seizures led to the death of 44% of animals that

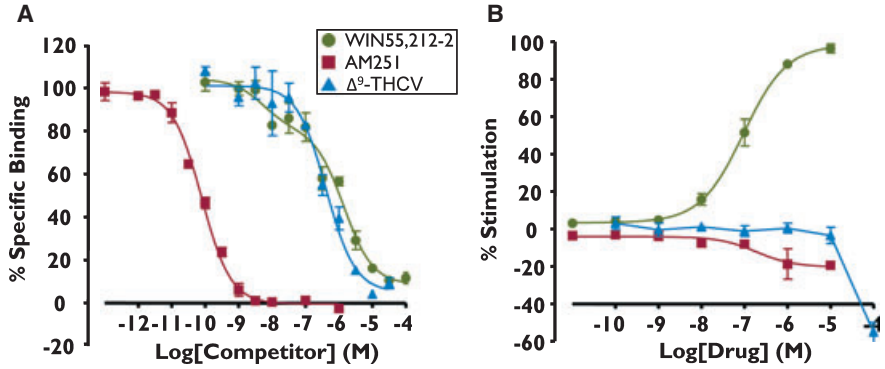

Figure 5.
Effects of Δ^9-tetrahydrocannabivarin (Δ^9-THCV), WIN55,212-2 (a synthetic, selective CB receptor antagonist), and AM251 (a synthetic, selective CB1receptor antagonist) on [^{3}H]SR141716A binding and [^{35}S]GTPγS binding assays in cortical membranes. (**A**) Representative competition curves for Δ^9-THCV, WIN55,212-2, and AM251 against 1 nM [^{3}H]SR141716A binding to cortical membranes. Δ^9-THCV displaces [^{3}H]SR141716A] binding with relatively high affinity. (**B**) Representative agonist binding curves for Δ^9-THCV, WIN55,212-2, and AM251 stimulation of [^{35}S]GTPγS binding to cortical membranes. Δ^9-THCV lacked agonist effects in [^{35}S]GTPγS binding assays in cortical membranes. In all cases, points are mean ± standard error of the mean (SEM) of triplicate points.
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received vehicle alone. Animals that received 0.025, 0.25, and 2.5 mg/kg Δ^9-THCV all exhibited lower mortality rates of 41%, 33%, and 38%, respectively; however, these values did not differ significantly from that of the vehicle group ($p > 0.05$, binomial test). In animals that experienced seizures, median seizure severity was unaffected by Δ^9-THCV treatment at any dose (Fig. 6A). Similarly, no effect of Δ^9-THCV on the duration, progression, or latency to the start of seizures was observed (data not shown). However, 33% of animals treated with 0.25 mg/kg Δ^9-THCV exhibited a complete absence of seizure behaviors (defined as no score above [0]). This percentage was significantly higher than that in animals treated with vehicle alone (13%; $p < 0.05$; Fig. 6B). This finding supports our in vitro findings and suggests that Δ^9-THCV exerts a dose-related anticonvulsant effect in the PTZ model of generalized seizures.

Discussion

In this study we demonstrate for the first time that Δ^9-THCV attenuates both in vitro epileptiform activity and can reduce in vivo seizure behavior. Therefore, acute application of Δ^9-THCV significantly reduced both the amplitude and frequency of established epileptiform bursting in rat PC in vitro (≥20 μM). Pretreatment with 10 μM Δ^9-THCV in the same model rendered epileptiform activity sensitive to a lower Δ^9-THCV concentration (10 μM) than

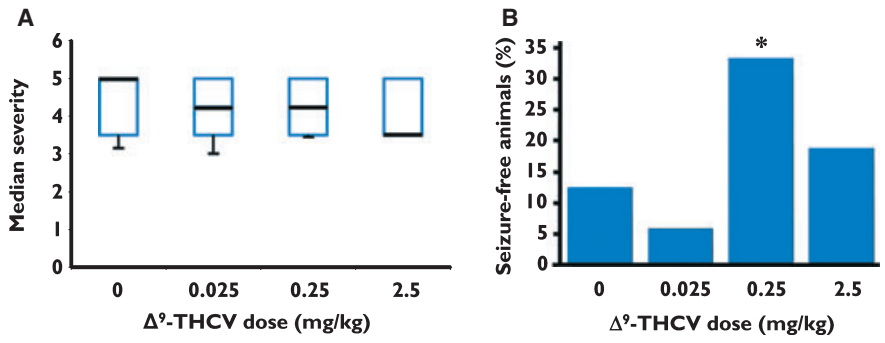

Figure 6.
Δ^9-tetrahydrocannabivarin (Δ^9-THCV) attenuates pentylenetetrazole (PTZ)–induced seizures. (**A**) Median seizure severity. Black lines show median severity, blue boxes show 25th and 75th percentiles, and error bars indicate 10th and 90th percentiles. (**B**) Percentage of seizure-free animals. n = 15 per group. *$p < 0.05$. Significance for seizure incidence % was assessed using a nonparametric binomial test.
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that seen in acutely treated slices. Δ^9-THCV (0.25 mg/kg) decreased the incidence of PTZ-induced seizures in adult rats in vivo. Furthermore, radioligand binding data indicate that, as has been previously reported in mouse cortical membranes (Dennis et al., 2008), Δ^9-THCV acts as a relatively high affinity CB1-receptor ligand in rat cortical brain membranes, a property that could underlie the antiepileptiform and anticonvulsant effects described.

Cannabis has been reported to exert both pro- and anticonvulsant effects, as has its principal psychoactive constituent Δ^9-THC (Chiu et al., 1979; Turkanis & Karler, 1981; Colasanti et al., 1982; Fish et al., 1983; Wallace et al., 2003). Δ^9-THC acts as a partial agonist at CB1 receptors (Shen & Thayer, 1999), implicating CB1 as a potential therapeutic target in epilepsy. However, assessment of the effects of other non-Δ^9-THC cannabinoid constituents of cannabis on epileptic seizures has thus far been limited to studies on cannabidiol (CBD) (Karler et al., 1974; Cunha et al., 1980; Consroe et al., 1982; Gordon & Devinsky, 2001; Wallace et al., 2001; Blair et al., 2009; Jones et al., 2010). Moreover, the broad range of pharmacologic targets and effects of the phytocannabinoids present in cannabis (Ben-Shabat et al., 1998), their varied proportions within the herb and between cannabis strains, and frequently additive or opposing effects (Pertwee et al., 2007) prevent straightforward conclusions being drawn from the effects of cannabis upon seizure states and epilepsy, which we begin to address here.

Δ^9-THCV exerts antiepileptiform effects in vitro

We report here that Δ^9-THCV attenuates epileptiform activity induced by Mg^{2+}removal in MEA recordings from PC slices following both acute application (40 μM Δ^9-THCV) and when applied prior to Mg^{2+}removal (10 μM Δ^9-THCV). These data demonstrate that pretreatment enhances the antiepileptiform effects of Δ^9-THCV in vitro. With regard to potential therapeutic application, this increased effect suggests that prophylactic use of Δ^9-THCV could enhance its potential as an antiepileptic drug (AED). Moreover, in addition to reducing PDS aptitude and frequency, Δ^9-THCV also inhibited PDS propagation across the PC slice. We reported recently that another prominent phytocannabinoid, CBD, similarly reduces epileptiform activity in Mg^{2+}-free conditions in hippocampal slices but, in contrast with Δ^9-THCV, CBD did not affect PDS propagation and had a much lower CB1-receptor affinity (Jones et al., 2010). It will clearly be of interest to determine if the action of phytocannabinoids with antiepileptic potential is dependent on brain region tested or on inherent mechanisms of action.

Competition assays confirmed a relatively high affinity interaction of Δ^9-THCV with CB1 receptors (K_i~290 nM) when compared with the very high affinity binding exhibited by the CB1-receptor antagonist AM251 (K_i~190 pM; Jones et al., 2010). Δ^9-THCV has also been reported to displace CB1-receptor agonist binding in whole brain membranes (Thomas et al., 2005; Pertwee et al., 2007) and to antagonize stimulation of [^{35}S]GTPγS binding by synthetic agonists in mouse whole brain, PC, and cerebellar membranes (Thomas et al., 2005; Pertwee et al., 2007; Dennis et al., 2008). Herein, we confirm our previous findings in mouse PC membranes by demonstrating that Δ^9-THCV had no stimulatory agonist effect on [^{35}S]GTPγS binding in rat cortex. Moreover, Δ^9-THCV exhibited CB1 receptor–independent inhibitory actions on [^{35}S]GTPγS binding and similar reductions in [^{35}S]GTPγS binding (Dennis et al., 2008) to those seen here. Overall, our binding data are consistent with Δ^9-THCV actions in the present study being mediated via CB1 receptors.

CB1 receptors and the endocannabinoid system as a whole play a crucial role in the development of the mammalian brain and regulation of its excitability. The endocannabinoid system is involved in specialization, migration, and neurite growth in the developing brain (Vitalis et al., 2008) and cannabis abuse during adolescence may result in altered cognitive performance and be a risk factor for adult schizophrenia (Rubino & Parolaro, 2008). In vivo electrophysiologic recordings from young (P5) rats indicate that endogenous CB1-receptor activation leads to decreased spontaneous neuronal activity, whereas inhibition induces epileptic discharges (Bernard et al., 2005). Evidence that the endocannabinoid system affects and is affected by epilepsy and seizures is abundant; CB1-receptor expression is downregulated in the brains of patients with intractable temporal lobe epilepsy (Ludanyi et al., 2008), whereas human neocortical AEA levels are elevated in epilepsy (Steffens et al., 2005). Furthermore, hippocampal CB1-receptor expression is decreased and redistributed in the pilocarpine model of chronic spontaneous recurrent seizures in rats, a change that may be involved in epileptogenesis in this model (Falenski et al., 2009). Moreover, inhibition of the enzyme responsible for AEA breakdown (fatty acid amide hydrolase; FAAH) has been reported to decrease the severity of kainate-induced seizures and associated neuronal death in rats by increasing brain AEA levels (Karanian et al., 2007). Consistent with evidence that CB1-receptor agonism generally attenuates seizures, cannabinoids and endocannabinoids block epileptiform activity in hippocampal neuronal culture models in vitro (Blair et al., 2006; Deshpande et al., 2007a), whereas CB1 antagonism causes status epilepticus–like activity in such models (Deshpande et al., 2007b). Importantly, in the hippocampus, endocannabinoid-mediated inhibition of epileptogenesis is associated with reductions in excitatory glutamate release (Monory et al., 2006; Ludanyi et al., 2008). Upon the basis of this evidence, it may be reasoned that CB1 receptor–mediated antagonism by Δ^9-THCV would potentiate Mg^{2+}-free aCSF-induced epileptiform activity in vitro. However, we have previously shown that Δ^9-THCV antagonizes endocannabinergic tone at GABAergic presynapses in acute

mouse cerebellar slices, increasing inhibitory neurotransmission and thus decreasing excitatory output from cerebellar Purkinje cells (Ma et al., 2008). If such preferential antagonism of presynaptic CB1-mediated action on inhibitory pathways were applicable to PC circuits crucial to epileptogenesis, then Δ^9-THCV would reduce hyperexcitability, consistent with the results presented herein. Other reports also indicate that CB1 antagonism can play a role in preventing or attenuating seizures. SR141716A, a CB1-receptor antagonist, prevented the increased susceptibility to kainate-induced seizures seen after head injury in rats if applied directly after injury (Echegoyen et al., 2009). Furthermore, low (sub-μg/kg) doses of AM251 significantly potentiated the anticonvulsant effects of the cannabinoid receptor agonist, arachidonyl-2-chloroethylamide (ACEA) (Kozan et al., 2009), and SR141716A-induced CB1 blockade produced significant neuroprotection following NMDA-induced excitotoxic neuronal damage (Hansen et al., 2002). Moreover, AEA-induced CB1-receptor activation caused $FAAH^{-/-}$ mice to suffer significantly worse chemically induced seizures than $FAAH^{+/+}$ animals (Clement et al., 2003), data apparently at odds with reports describing FAAH inhibition as anticonvulsant (Karanian et al., 2007). These findings likely reflect descriptions of both pro- and anticonvulsant properties of cannabinoids, and further substantiate the hypothesis that receptor localization to excitatory versus inhibitory presynaptic terminals within the CNS dictates functional effects of CB1 receptor ligands (Puighermanal et al., 2009). Consequently, the specific mechanisms underlying epileptogenesis, maintenance, and spread in the specific models used are likely to contribute substantially to the overall pro- or antiepileptiform effect of CB1 antagonists such as Δ^9-THCV.

The present study also suggests that, despite nanomolar CB1-receptor affinities (see also Thomas et al., 2005; Dennis et al., 2008), it is necessary to use relatively high concentrations of highly lipophilic cannabinoids in brain slice investigations (Brown et al., 2004; Ma et al., 2008). Therefore, it is even more notable that lower Δ^9-THCV pretreatment concentrations (10 μM) were required to produce antiepileptiform effects. Given that the pretreatment rationale used here may be argued as closer to the prophylactic use of anticonvulsants in the clinic, the low concentrations of Δ^9-THCV required to induce antiepileptiform effects could support a potential therapeutic use.

Δ^9-THCV exerts antiseizure effects in vivo

In our in vivo seizure experiments, 0.25 mg/kg Δ^9-THCV exerted a modest anticonvulsant effect by attenuating seizure incidence. We propose that Δ^9-THCV antiepileptiform effects in vitro are mediated, at least in part, by an action at CB1 receptors and, in this regard, it is possible that preferential antagonism of endocannabinergic tone at GABAergic presynapses (Ma et al., 2008) is implicated. However, this mechanism is very unlikely to underlie the anticonvulsant Δ^9-THCV properties in vivo reported here, as PTZ acts as a $GABA_A$ antagonist (Zhao & Holmes, 2006). Nevertheless, it is feasible that Δ^9-THCV may sufficiently disrupt the neuronal synchronization necessary for seizure activity by affecting the balance of endocannabinergic tone at glutamatergic and GABAergic presynapses (Weston et al., 2007; Mason & Cheer, 2009). Furthermore, as has also been reported for other phytocannabinoids, Δ^9-THCV exerts pharmacologic effects via non–CB1-mediated mechanisms (Pertwee, 2008), which cannot presently be excluded from possible mechanisms underlying effects in vivo.

However, there remains a contradiction between CB1 agonist and antagonist effects upon hyperexcitability states. In contrast to the anticonvulsant effects of CB1 antagonism in vivo presented in this study, CB1 activation in vivo has been shown to reduce seizure susceptibility in a range of rodent models (Wallace et al., 2001, 2002, 2003; Mason & Cheer, 2009; Rizzo et al., 2009), including the PTZ model used in this study (Shafaroodi et al., 2004; Bahremand et al., 2008). Moreover, the anticonvulsant properties of valproate (Luszczki et al., 2006) and diazepam (Naderi et al., 2008) are enhanced by CB1-receptor activation, suggesting potentially synergistic antiseizure activity. As discussed earlier, it may be speculated that CB1 receptor–mediated effects are dependent on relative expression levels at glutamatergic versus GABAergic presynapses.

It is also noteworthy that Δ^9-THCV has a clearly biphasic dose–response effect. For example, synthetic Δ^9-THCV exerts a dose-dependent antagonist action in tail-flick assays in vivo, but at higher doses has agonist actions (Pertwee et al., 2007). It has been proposed that this may be attributed to in vivo metabolism, although it remains to be seen whether the anticonvulsant effects of Δ^9-THCV in PTZ-induced seizures reported herein represent an in vivo manifestation of such biphasic concentration–response results and/or in vivo metabolism.

Conclusions

This study demonstrates for the first time that Δ^9-THCV possesses antiepileptiform properties in the PC in vitro, a brain area prone to epileptogenesis, likely via an interaction with CB1 receptors. Furthermore, Δ^9-THCV caused a dose-related reduction in seizure incidence in an in vivo model of generalized seizures. We conclude that Δ^9-THCV is a phytocannabinoid that warrants further investigation as a potentially useful anticonvulsant, either as a standalone agent or more likely as an adjunct to conventional AEDs.

Acknowledgments

The authors would like to thank GW Pharmaceuticals Ltd (United Kingdom) and Otsuka Pharmaceutical Ltd (Japan) for provision of Δ^9-THCV

and funding. IS was funded by the University of Reading Research Endowment Trust Fund. We thank Professor P. G. Strange for his invaluable support and assistance with radioligand assays and Mr C. Stott (GW Pharmaceuticals Ltd (United Kingdom) and Prof. G. Riedel (University of Aberdeen, United Kingdom) for kind provision of pharmacokinetic and toxicologic data. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Disclosure

Andrew J. Hill, Nicholas A. Jones, Gary J. Stephens, Claire M. Williams, and Benjamin J. Whalley received support from GW Pharmaceuticals and Otsuka Pharmaceuticals. Imogen Smith received support from the University of Reading Endowment Trust Fund.

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