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RESEARCH PAPER

Cannabidiol inhibits synaptic transmission in rat hippocampal cultures and slices via multiple receptor pathways

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BACKGROUND AND PURPOSE
Cannabidiol (CBD) has emerged as an interesting compound with therapeutic potential in several CNS disorders. However, whether it can modulate synaptic activity in the CNS remains unclear. Here, we have investigated whether CBD modulates synaptic transmission in rat hippocampal cultures and acute slices.

EXPERIMENTAL APPROACH
The effect of CBD on synaptic transmission was examined in rat hippocampal cultures and acute slices using whole cell patch clamp and standard extracellular recordings respectively.

KEY RESULTS
Cannabidiol decreased synaptic activity in hippocampal cultures in a concentration-dependent and *Pertussis* toxin-sensitive manner. The effects of CBD in culture were significantly reduced in the presence of the cannabinoid receptor (CB_1) inverse agonist, LY320135 but were unaffected by the 5-HT_{1A} receptor antagonist, WAY100135. In hippocampal slices, CBD inhibited basal synaptic transmission, an effect that was abolished by the proposed CB_1 receptor antagonist, AM251, in addition to LY320135 and WAY100135.

CONCLUSIONS AND IMPLICATIONS
Cannabidiol reduces synaptic transmission in hippocampal *in vitro* preparations and we propose a role for both 5-HT_{1A} and CB_1 receptors in these CBD-mediated effects. These data offer some mechanistic insights into the effects of CBD and emphasize that further investigations into the actions of CBD in the CNS are required in order to elucidate the full therapeutic potential of CBD.

Abbreviations
aCSF, artificial cerebrospinal fluid; AP5, (2R)-amino-5-phosphonovaleric acid; CB_1, cannabinoid receptor; CBD, cannabidiol; DIV, days *in vitro*; fEPSP, field excitatory postsynaptic potentials; GPCR, G-protein coupled receptor; HBS, HEPES-buffered saline; NBQX, 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulphonamide; 8-OH-DPAT, 8-hydroxy-2(di-N-propylamino)tetralin; THC, Δ^9-tetrahydrocannabinol

Introduction

The Asian hemp plant, *Cannabis sativa*, has been used therapeutically for centuries but until recently most research has focused on Δ^9-tetrahydrocannabinol (THC), the most abundant phytocannabinoid in cannabis. THC, acting via the cannabinoid CB_1 or CB_2 receptors (nomenclature follows Alexander *et al.*, 2009), is thought to underlie most of the therapeutic uses of cannabis, which include use as an analgesic, an antiepileptic and an antiemetic (see Ben Amar, 2006; Turcotte *et al.*, 2010). However, there remains controversy over the therapeutic use of cannabis because of the adverse psychotomimetic effects of THC and its potential to cause cognitive impairments (Fletcher and Honey, 2006; D'Souza *et al.*,

2008). Nevertheless, cannabis contains numerous cannabinoids and these different constituents may modulate the effects of THC and have useful effects of their own. This has led to intense investigation into the therapeutic potential of other, non-psychoactive, constituents of cannabis (Russo and Guy, 2006; Pertwee, 2008; Izzo *et al.*, 2009).

In particular cannabidiol (CBD), which lacks negative psychotropic effects when administered to humans even in high doses (Bhattacharyya *et al.*, 2009), has emerged as an interesting compound due to its potential therapeutic application in a number of neurological and neuropsychiatric disorders including addiction, diabetic neuropathy, epilepsy, schizophrenia and stroke and it is currently used at a 1:1 ratio with THC in Sativex® for the treatment of chronic pain in patients suffering from multiple sclerosis and advanced cancer (see Russo and Guy, 2006; Izzo *et al.*, 2009). Despite the fact that CBD is used clinically, the exact mechanism(s) by which it exerts its therapeutic action remains unclear, although several targets have been identified which could underlie its therapeutic actions in CNS disorders (see Russo and Guy, 2006; Pertwee, 2008 and Izzo *et al.*, 2009). In contrast to THC, CBD has a low affinity for CB_1 receptors and acts as a CB_1 receptor antagonist and a CB_2 receptor inverse agonist (Showalter *et al.*, 1996; Bisogno *et al.*, 2001; Thomas *et al.*, 2007). Furthermore, CBD has been proposed as an agonist at 5-HT_{1A} receptors (Russo *et al.*, 2005) and this has been suggested to mediate the anxiolytic and anti-ischaemic properties of CBD (Campos and Guimarães, 2008; Resstel *et al.*, 2009). CBD also acts either as an agonist or antagonist at transient receptor potential cation channels (TRPs; De Petrocellis *et al.*, 2008; Qin *et al.*, 2008) and has also been shown to inhibit the Ca_v3 subfamily of Ca^{2+} channels (Ross *et al.*, 2008). In addition, CBD can modulate intracellular Ca^{2+} levels when tested on cultured hippocampal neurons, an effect proposed to be mediated via Ca_v1 Ca^{2+} channels, Ca^{2+} release from intracellular stores and the modulation of mitochondrial function (Drysdale *et al.*, 2006; Ryan *et al.*, 2009).

Despite increasing evidence to suggest that CBD has multiple targets (Pertwee, 2008; Izzo *et al.*, 2009), the effect of CBD on basal synaptic transmission has yet to be determined although a recent study has demonstrated clear anti-epileptiform and antiseizure activity (Jones *et al.*, 2010). In the present study, we have examined the effect of CBD upon synaptic activity in primary hippocampal cultures and acute hippocampal slices. Our data show for the first time that CBD is able to modulate basal hippocampal synaptic activity and we suggest a role for CB_1 and 5-HT_{1A} receptors in these observations. These data provide some mechanistic insights into the effects of CBD and highlight that further investigations into the actions of CBD in the CNS are required in order to fully elucidate the contribution of CBD to the detrimental and therapeutic roles of cannabis.

Methods

Primary hippocampal cultures

All animal care and experimental procedures were in accordance with UK Home Office guidelines. Primary hippocampal cultures were prepared as described previously (Greenwood *et al.*, 2007). Briefly, 1- to 2-day-old Sprague Dawley rats were killed by cervical dislocation and decapitated. Once the hippocampi were removed and triturated, cells were plated at a density of 3×10^5 cells·mL^{-1} onto poly-L-lysine coated coverslips. Cultures were incubated in a medium consisting of Neurobasal-A Medium (Invitrogen, Paisley, UK) supplemented with 2% (v/v) B-27 (Invitrogen) and 2 mM L-glutamine and maintained in a humidified atmosphere at 37°C/5% CO_2 for 13–16 days *in vitro* (DIV). After 5 DIV, cytosine-D-arabinofuranoside (10 μM) was added to inhibit glial cell proliferation. All experiments were performed on cells taken from at least three separate cultures obtained from different rats.

Hippocampal slice preparation

Sprague-Dawley rats (16–20 days old) were killed by cervical dislocation and decapitated. The brains were rapidly removed and placed immediately in an ice-cold (0–3°C), oxygenated (95% O_2/5% CO_2) cutting solution containing (in mM): sucrose 26, $NaHCO_3$ 2, NaH_2PO_4 2, $MgSO_4$ 3, KCl 2, $CaCl_2$ and D-glucose 10. Parasagittal whole brain slices (400 μm) were cut using a vibratome and placed into oxygenated artificial cerebrospinal fluid (aCSF) with the same composition as the cutting solution, but with NaCl (124 mM) replacing the sucrose. Hippocampal regions were then dissected free and placed in a submerged holding chamber containing aCSF continuously bubbled with 95% O_2/5% CO_2. Slices were allowed to equilibrate for a minimum of 1 h at room temperature prior to use.

Electrophysiology

Cultured neurones. Cells were perfused at 2 mL·min^{-1} with a HEPES-buffered saline (HBS) containing (in mM): NaCl 140, KCl 5, $MgCl_2$ 2, $CaCl_2$ 2, HEPES 10, D-glucose 10, pH was adjusted to 7.4 and osmolarity adjusted to 310 mOsm with sucrose if required. Current clamp recordings were made using whole cell patch clamp in current clamp mode with glass pipettes (4–6 MΩ) filled with a internal

solution containing (in mM): KCl 150, $MgCl_2$ 1, $CaCl_2$ 1, HEPES 10, EGTA 0.5, Mg-ATP 3, GTP 0.3, pH was adjusted to 7.2 and osmolarity was adjusted to 290 mOsm using sucrose if required. Data were acquired with an Axopatch-200B amplifier (Molecular Devices, Sunnyvale, CA, USA) and using WinEDR v 2.7.9 software (J. Dempster, University of Strathclyde, Glasgow, UK). No capacitance and series resistance compensation were applied. All drugs were added via the perfusate. In experiments where CBD alone was applied, it was added for 5 min following a 5 min steady baseline period and then washed out with perfusate. In experiments where antagonists were used, antagonists at the stated concentration were applied for 5 min following a 5 min baseline period. Subsequently, antagonist + CBD were added together for a further 5 min period. Finally, all drugs were washed out with perfusate. Data were analysed offline using WinEDR v 2.7.9.

Acute slice electrophysiology. Slices were transferred to a submerged recording chamber continually perfused with oxygenated aCSF at a flow rate of 1–2 mL·min^{-1}. Extracellular field excitatory postsynaptic potential (fEPSP) recordings were obtained in response to low frequency (0.033 Hz) stimulation of the Schaffer collateral-commissural pathway by a bipolar stimulating electrode and a borosilicate glass recording electrode filled with 4 M NaCl placed in the stratum radiatum of area CA1. Paired-pulse experiments were performed with an inter-stimulus interval of 50 ms. All data were acquired using an Axopatch-2B amplifier (Molecular Devices) and using WinWCP v 2.7.9 software (J. Dempster, University of Strathclyde, Glasgow, UK) with the fEPSP slope analysed online and reanalysed offline using WinWCP v 2.7.9.

Statistical analysis

All data are expressed as mean ± SEM. Data were compared by paired or unpaired Student's *t*-tests, or one-way analysis of variance with Tukey's comparison as appropriate with $P < 0.05$ considered significant.

Materials

All chemicals were obtained from Sigma-Aldrich (Poole, UK) or VWR (East Grinstead, UK) except for THC and CBD (Tocris, Bristol, UK or THC Pharma, Frankfurt, Germany), AM251, DL-(2*R*)-amino-5-phosphonovaleric acid (AP5) and 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulphonamide (NBQX) (Ascent Scientific, Bristol, UK) and 8-hydroxy-2(di-N-propylamino)tetralin (8-OH-DPAT), LY320135, WAY100135 and WIN 55,212-2 (Tocris).

Results

Cannabidiol reduces spontaneous action potential frequency in cultured hippocampal neurones

Application of CBD reduced the synaptically driven spontaneous action potential (AP) frequency observed in primary hippocampal cultures in a concentration-dependent manner (0.1 μM, no significant change; 1 μM, 88 ± 8% decrease; 10 μM, 100% decrease; all $n = 5$, $P < 0.001$, Figure 1A, B).

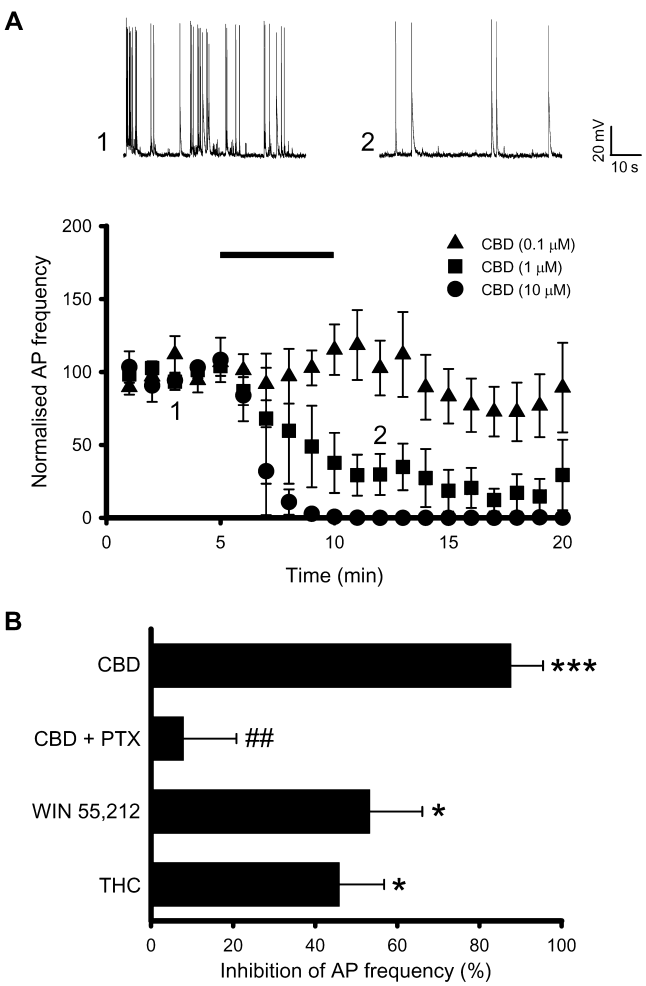

Figure 1

Cannabidiol (CBD) decreases spontaneous action potential (AP) firing frequency in hippocampal cultures, in a concentration-dependent and *Pertussis* toxin (PTX)-sensitive manner. (A) Time course revealing that AP firing frequency is reduced by CBD (1 & 10 μM). Representative traces illustrating spontaneous AP firing in the absence and presence of CBD (1 μM) are shown for the time points indicated. (B) Summary data showing that AP firing frequency in primary hippocampal cultures was reduced by CBD (1 μM) in a PTX-sensitive manner and by the cannabinoid receptor agonists, WIN 55,212 and Δ^9-tetrahydrocannabinol (THC). *$P < 0.05$, ***$P < 0.001$, ##$P < 0.01$ compared with CBD alone.

Confirmation of the synaptically driven nature of the spontaneous APs was shown by their sensitivity to the ionotropic glutamate receptor antagonists, DL-AP5 (100 μM) and NBQX (20 μM) (data not shown). In contrast, CBD was without effect on the resting membrane potential (V_m) at all concentrations tested (0.1 μM, 0.2 ± 2.1 mV from a control V_m of −60.7 ± 2.1 mV; 1 μM, 1.5 ± 1.6 mV from a control V_m of −57.6 ± 1.6 mV; 10 μM, 0.3 ± 0.7 from a control V_m of −62.0 ± 3.7 mV; all $n = 5$ and $P > 0.05$). Similar reductions in spontaneous AP frequency were observed following the application of the CB_1 agonist, WIN 55,212-2 (0.1 μM Figure 1B) and the CB_1 partial agonist, THC (10 μM, Figure 1B), as has been shown previously (Shen and Thayer, 1999; Bajo *et al.*, 2009; Roloff and Thayer, 2009). To determine if the effects of CBD were mediated by a $G\alpha_{i/o}$ G-protein coupled receptor (GPCR), experiments were performed on cultures treated with *Pertussis* toxin (PTX, 200 ng·mL^{-1}, 18 h). The CBD-induced reduction in spontaneous AP frequency was abolished following PTX treatment, compared to CBD alone (1 μM, Figure 1B).

In order to examine whether the CBD-induced effects involved CB_1 receptor activation, we utilized the proposed CB_1 receptor antagonist AM251 and the structurally dissimilar CB_1 receptor inverse agonist LY320135. Application of AM251 (30 nM) resulted in an initial dramatic increase in AP firing rate, presumably due to the inhibition of the endogenous cannabinoid tone, that in the majority of cases led to a significant depolarization of the membrane potential and, as a consequence, to a loss of AP firing (Figure 2A). Thus the effect of AM251 on CBD-induced effects could not be investigated in our culture system. In contrast, application of LY320135 (1 μM) by itself had no significant effect on the AP firing frequency (14.9 ± 4.2% decrease, $n = 7$) but did significantly inhibit the decrease in AP firing frequency observed following CBD (1 μM) application, compared to CBD alone (Figure 2C). With CBD proposed as a 5-HT_{1A} receptor agonist, we examined in our culture preparation whether the 5-HT_{1A} receptor was involved in the CBD-mediated effects. Application of the 5-HT_{1A} receptor agonist, 8-hydroxy-2(di-N-propylamino)tetralin (8-OH-DPAT, 10 μM) significantly reduced the spontaneous AP frequency (Figure 2B, C) without affecting the V_m (1.2 ± 1.3 mV decrease from a control V_m of −59.4 ± 1.2, $n = 5$). The ability of 8-OH-DPAT to reduce spontaneous AP frequency was significantly inhibited in the presence of the 5-HT_{1A} receptor antagonist, WAY100135 (300 nM), compared with 8-OH-DPAT alone (Figure 2C). However, WAY100135 (300 nM) had no effect on CBD-mediated inhibition (1 μM) (Figure 2C), suggesting that in our culture system, the effects of CBD and 8-OH-DPAT were mutually exclusive.

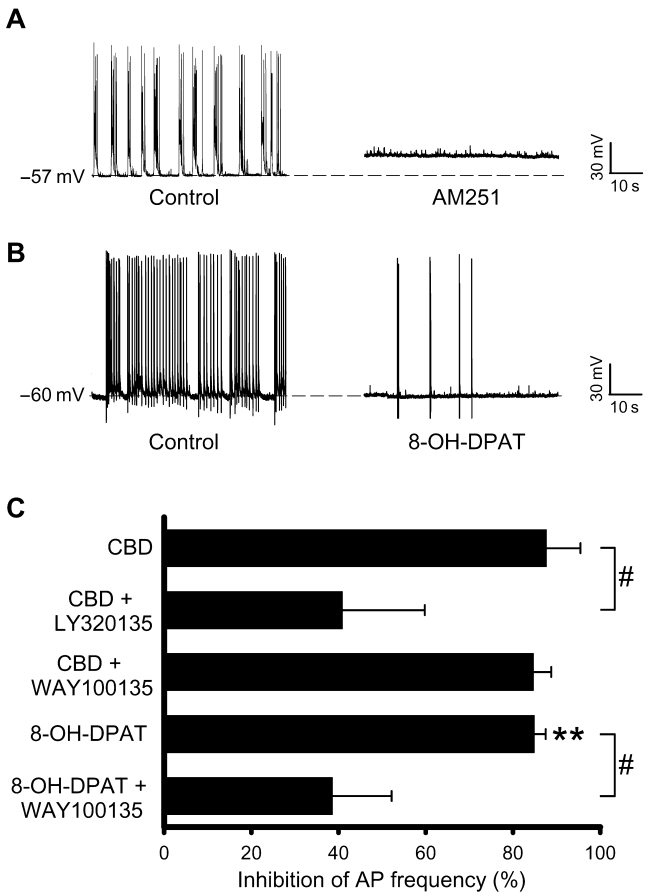

Figure 2

Cannabidiol (CBD)-mediated decrease in action potential (AP) firing frequency in hippocampal cultures is sensitive to the cannabinoid receptor inverse agonist, LY320135. (A) Representative traces illustrating the depolarization and consequent elimination of spontaneous APs observed following the application of AM251 (30 nM). (B) Representative traces showing the reduction in AP firing in the presence of the 5-HT_{1A} agonist, 8-OH-DPAT. (C) Summary data showing the effect of LY320135 and WAY100135 on CBD and 8-OH-DPAT-mediated inhibition of AP firing. **$P < 0.01$, # $P < 0.05$ compared with CBD or 8-OH-DPAT alone.

Cannabidiol inhibits synaptic transmission in hippocampal slices

Having established the effect of CBD application in hippocampal cultures, we next investigated whether CBD modulates synaptic transmission in acute hippocampal slices. Application of CBD (10 μM) reversibly inhibited fEPSPs by 39 ± 13% ($n = 6$, $P < 0.05$, Figure 3A). The CBD-induced inhibitions were associated with a significant increase in the fEPSP paired-pulse facilitation ratio which increased from the control ratio of 1.65 ± 0.07 to 2.09 ± 0.13 in the presence of CBD ($n = 6$, $P < 0.01$, Figure 3B). This

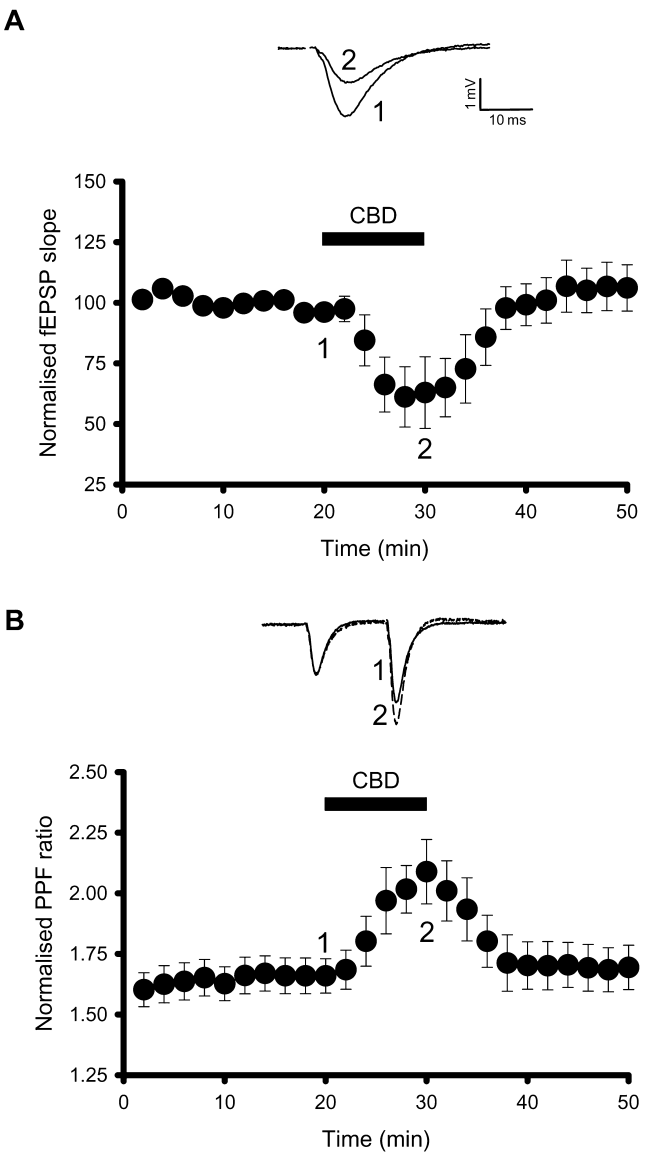

Figure 3

Cannabidiol (CBD) inhibits synaptic transmission in acute slices from the CA1 region of the hippocampus. (A) CBD (10 μM) application reversibly inhibits field excitatory postsynaptic potentials (fEPSP). Representative fEPSP traces are shown for the time points indicated. (B) CBD inhibition of synaptic transmission is associated with an increase in paired-pulse facilitation (PPF) ratio. Scaled fEPSP traces from (A) are shown to demonstrate the increase in paired-pulse facilitation.

finding, aligned with the PTX sensitivity of the CBD effects observed in primary cultures, suggest that a presynaptic GPCR underlies the CBD-mediated inhibition of synaptic transmission. As in experiments in the culture preparation, we utilized the proposed CB_1 receptor antagonist, AM251 and the CB_1 receptor inverse agonist LY320135 in our slice preparation to investigate the involvement of CB_1 receptors. To our surprise, both AM251 (2 μM) and LY320135 (1 μM) abolished the inhibition of synaptic transmission by CBD (Figure 4A, E). Despite the lack of effect of the 5-HT_{1A} receptor antagonist WAY100135 on CBD-mediated effects in cultures, we also investigated the role of 5-HT_{1A} receptors in the CBD-mediated effects on synaptic transmission in the slice preparation. Strikingly, WAY100135 (300 nM) abolished the CBD mediated inhibition of synaptic transmission (Figure 4B, E). In addition and similar to its effect in cultures, 8-OH-DPAT (10 μM), like CBD, significantly inhibited basal synaptic transmission (Figure 4C, E). However, in contrast to CBD, it was without effect on the fEPSP paired-pulse facilitation ratio (control 1.71 ± 0.11, 8-OH-DPAT 1.73 ± 0.13). With evidence existing for AM251 acting via non-CB_1 receptor mechanisms (Pertwee, 2005), the specificity of the two putative CB_1 receptor inhibitors was investigated against 8-OH-DPAT-mediated inhibitions. Intriguingly, AM251 (2 μM) significantly inhibited the 8-OH-DPAT (10 μM)-induced depression (Figure 4D, E) whereas LY320135 (1 μM) was without effect (Figure 4E).

Discussion

The physiological actions of CBD, a purported non-psychoactive constituent of cannabis, have recently been an area of intense interest especially as it has been proposed to be beneficial in a number of clinical settings. In the present study, we show for the first time that CBD inhibits basal synaptic activity in both hippocampal cultures and acute slices.

Unlike THC, whose physiological effects are thought to be mediated predominantly through CB receptors, CBD has been shown to modulate a multitude of signalling mechanisms (Russo and Guy, 2006; Pertwee, 2008; Izzo *et al.*, 2009). Utilizing *in vitro* CNS preparations, CBD has been shown to increase intracellular Ca^{2+} levels when investigated in cultured hippocampal neurons, an effect proposed to be mediated via Ca_v1 Ca^{2+} channels, Ca^{2+} release from intracellular stores and the modulation of mitochondrial function (Drysdale *et al.*, 2006; Ryan *et al.*, 2009). However, these studies were not extended to include the effect of CBD on synaptic activity although CBD did reduce intracellular Ca^{2+} levels under conditions of high neuronal excitability or epileptiform activity (Ryan *et al.*, 2009), a finding supported by a recent study which shows that CBD has anti-epileptiform activity in hippocampal slice models of epilepsy (Jones *et al.*, 2010). Our findings suggest a mechanism by which CBD inhibits synaptic activity in hippocampal culture preparations. We propose that CBD acts via a $G\alpha_{i/o}$

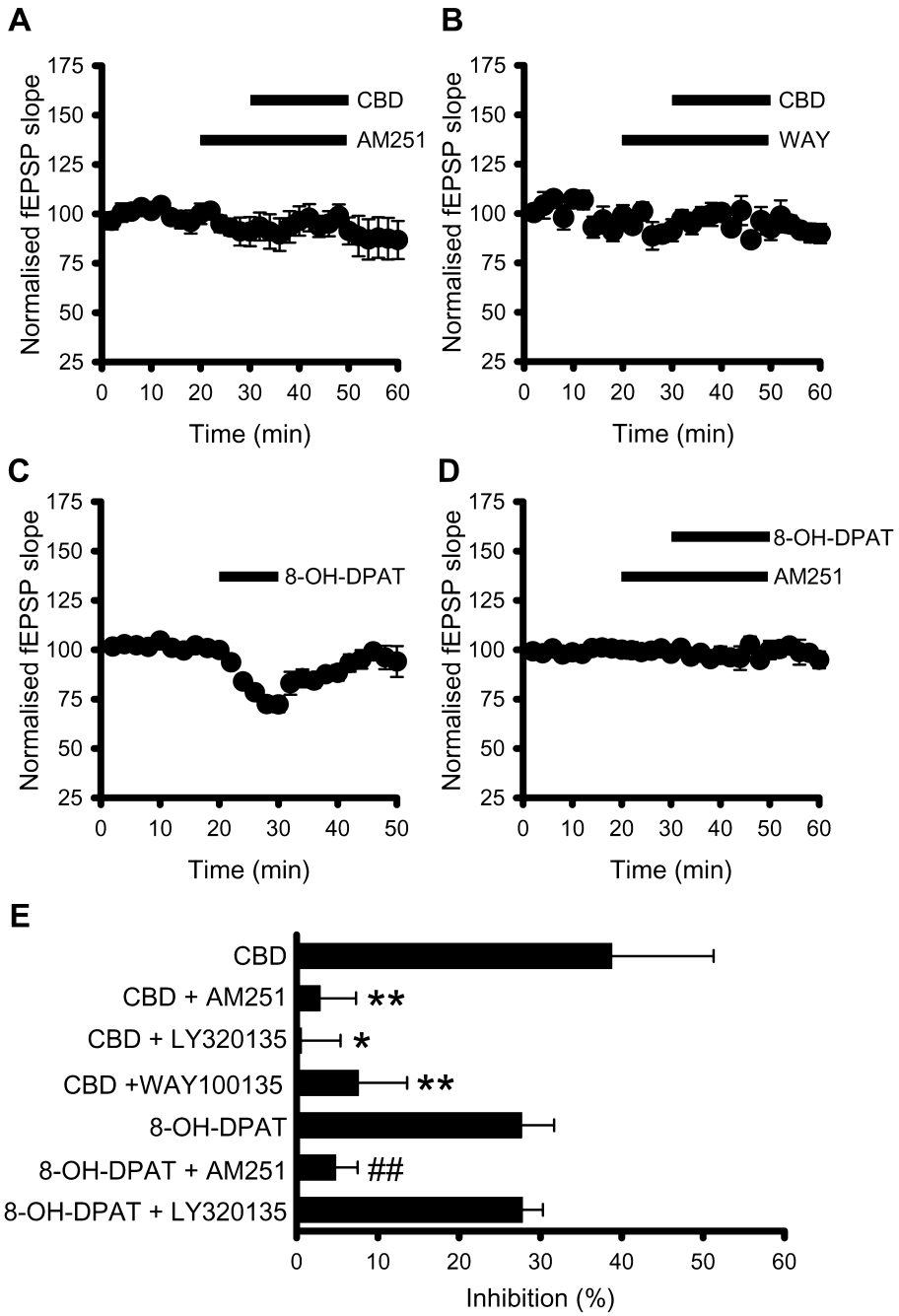

Figure 4

Cannabidiol (CBD)-mediated inhibition of synaptic transmission in acute hippocampal slices is sensitive to 5-HT_{1A} and cannabinoid receptor (CB_1) antagonism. (A) CBD (10 μM)-mediated inhibition of synaptic transmission was abolished by the proposed CB_1 receptor antagonist AM251 (2 μM). (B) Inhibition of transmission by CBD was significantly reduced in the presence of the 5-HT_{1A} receptor antagonist, WAY100135 (300 nM). (C) 8-OH-DPAT (10 μM) reversibly inhibits field excitatory postsynaptic potentials (fEPSP). (D) AM251 (2 μM) inhibits the 8-OH-DPAT (10 μM)-mediated inhibition of fEPSPs. (E) Summary data showing that CBD-mediated effects were significantly reduced in the presence of 5-HT_{1A} and CB_1 receptor antagonists. *P < 0.05, **P < 0.01 compared with CBD alone. ##P < 0.01 compared with 8-OH-DPAT alone.

coupled GPCR as its effect was abolished following pre-incubation with the $G\alpha_{i/o}$ G-protein uncoupler, PTX. However, as spontaneous APs observed in our investigation are synaptically driven and with CBD decreasing intracellular Ca^{2+} levels in the neuronal soma under certain conditions (Ryan *et al.*, 2009), a direct action of CBD on presynaptically located Ca^{2+} channels or their inhibition through the activation of presynaptic GPCRs cannot be unequivocally ruled out. Indeed, an inhibition of Ca_v3-type Ca^{2+}

channels by CBD has been shown in heterologous expression systems and sensory neurones (Ross *et al.*, 2008). However, as this Ca^{2+} channel subtype is not thought to be involved in neurotransmitter release within the hippocampus (Wheeler *et al.*, 1994; Catterall, 2000), the action of CBD on this channel subtype probably does not underlie its effect in the current study. Moreover, modulation of intracellular stores and mitochondria has also been shown to affect synaptic transmission in CNS preparations (Billups and Forsythe, 2002; Fitzjohn and Collingridge, 2002; Le Magueresse and Cherubini, 2007); however, their potential role in the CBD-mediated effects observed here are beyond the focus of the present study.

As THC and WIN 55,212-2 both decreased synaptic activity in agreement with previous studies (Shen and Thayer, 1999; Ohno-Shosaku *et al.*, 2005; Straiker and Mackie, 2005; Bajo *et al.*, 2009), this indicates that the CBD-mediated effects are not an artefact of our culture preparation. This was further verified by observations that CBD inhibited basal synaptic transmission and increased the paired-pulse facilitation ratio in acute hippocampal slices. An increase in paired-pulse facilitation ratio is indicative that CBD is acting via a presynaptic receptor (Baskys and Malenka, 1991) and this finding, aligned to the sensitivity to PTX of the CBD-mediated actions in hippocampal cultures, suggested that the actions of CBD were mediated, at least in part, via a presynaptic $G\alpha_{i/o}$ coupled GPCR. Hence we investigated the potential role, or lack thereof, of CB_1 receptors in the CBD-mediated effects. To our initial surprise, we observed a decrease in the CBD-mediated effects in slices by the proposed CB_1 receptor antagonist AM251 and the structurally dissimilar CB_1 receptor inverse agonist LY320135. As mentioned earlier, CBD is proposed to have a low affinity for CB_1 receptors with no agonist activity and potentially act as a CB_1 receptor antagonist (Showalter *et al.*, 1996; Bisogno *et al.*, 2001; Thomas *et al.*, 2007; Jones *et al.*, 2010). It is therefore unlikely that the observed CBD effects are mediated through a direct activation of CB_1 receptors. However, an alternative explanation may account for the effects seen with AM251 and LY320135. As CBD has been shown to inhibit the reuptake and metabolism of the endocannabinoid anandamide (Rakhshan *et al.*, 2000; Bisogno *et al.*, 2001), the application of CBD could lead to an increase in the endocannabinoid tone and thus lead to a decrease in synaptic transmission via CB_1 receptor activation, as has been shown previously for exogenously applied endocannabinoids in culture (Straiker and Mackie, 2005; Hashimotodani *et al.*, 2007) and in slices under conditions where degradation was inhibited (Bajo *et al.*, 2009). It should be noted, however, that care needs to be taken when interpreting the results obtained using AM251 as this proposed selective CB_1 receptor antagonist abolished the effect of 8-OH-DPAT in the present study, indicating non-CB_1 receptor effects, as proposed previously (Pertwee, 2005). Despite this, the results obtained with LY320135 do indeed suggest a role for CB_1 receptors in the CBD-mediated effects. Taken together, these data imply the direct activation of $5\text{-}HT_{1A}$ receptors and the indirect activation of CB_1 receptors in the CBD-mediated effects. However, other signalling mechanisms previously shown to be modulated by CBD may also contribute to the observed effects and cannot be excluded.

An area of current interest regarding the actions of CBD is its proposed action as a putative agonist at the 5-HT receptor subtype, $5\text{-}HT_{1A}$ (Russo *et al.*, 2005). Other studies also support the involvement of $5\text{-}HT_{1A}$ receptors in CBD-mediated effects as $5\text{-}HT_{1A}$ receptor antagonists have been shown to block the anxiolytic effects of CBD observed in animal models of anxiety, depression and stress (Campos and Guimarães, 2008; Resstel *et al.*, 2009; Zanelati *et al.*, 2010). In our experiments, WAY100135 abolished the inhibitory effects of CBD on basal synaptic transmission in acute slices implicating $5\text{-}HT_{1A}$ receptors in the CBD-mediated effects observed here. In agreement with this, activation of $5\text{-}HT_{1A}$ receptors with 8-OH-DPAT inhibited basal hippocampal synaptic transmission, as shown in previous studies (Schmitz *et al.*, 1995; 1998; Pugliese *et al.*, 1998). This raises the possibility that CBD could potentially modify hippocampal-dependent behaviours through the activation of $5\text{-}HT_{1A}$ receptors. However, the effects of $5\text{-}HT_{1A}$ receptor activation on synaptic transmission in acute slices have been proposed to be mediated via postsynaptic activation of G-protein-coupled inwardly rectifying K^+ channels (Andrade and Nicoll, 1987; Lüscher *et al.*, 1997) and the lack of effect of 8-OH-DPAT on fEPSP paired-pulse ratio in the current study is consistent with this. It should be noted that 8-OH-DPAT reduced spontaneous AP firing in cultures without affecting the membrane potential. 5-HT receptor-mediated K^+ channel activation has been shown to run down in cultures in whole cell mode (Yakel *et al.*, 1988) whilst differences between cultures and slices have been reported previously with regard to the K^+ channel type involved following 5-HT receptor activation (Premkumar and Gage, 1994). As our culture experiments are in whole cell mode and are performed only when a stable firing frequency has been obtained, we suggest this is the explanation for the lack of effect on membrane potential seen with 8-OH-DPAT. However, further investigation into the

disparity on the membrane potential effects between culture and slice preparations is beyond the scope of this study.

In conclusion, we have shown that CBD reduces synaptic activity in *in vitro* hippocampal preparations and propose a role for the direct activation of 5-HT_{1A} receptors along with an indirect activation of CB_1 receptors in these CBD-mediated effects. These data offer some clues into the mechanisms through which CBD may be reducing regional brain activity (Fusar-Poli *et al.*, 2009; Bhattacharyya *et al.*, 2010) and epileptiform activity (Jones *et al.*, 2010) as reported in recent studies and suggest that further investigations into the actions of CBD in the CNS are required in order to fully elucidate its therapeutic potential.

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Conflicts of interest

There are no conflicts of interest regarding the content of this manuscript.

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