Neurochemical Research
https://doi.org/10.1007/s11064-018-2513-z

ORIGINAL PAPER

Cannabidiol Reverses Deficits in Hippocampal LTP in a Model of Alzheimer's Disease

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Received: 15 December 2017 / Revised: 16 March 2018 / Accepted: 20 March 2018

Abstract
Here we demonstrate for the first time that cannabidiol (CBD) acts to protect synaptic plasticity in an in vitro model of Alzheimer's disease (AD). The non-psycho active component of *Cannabis sativa*, CBD has previously been shown to protect against the neurotoxic effects of beta amyloid peptide (Aβ) in cell culture and cognitive behavioural models of neurodegeneration. Hippocampal long-term potentiation (LTP) is an activity dependent increase in synaptic efficacy often used to study cellular mechanisms related to memory. Here we show that acute application of soluble oligomeric beta amyloid peptide ($A\beta_{1–42}$) associated with AD, attenuates LTP in the CA_1 region of hippocampal slices from C57Bl/6 mice. Application of CBD alone did not alter LTP, however pre-treatment of slices with CBD rescued the $A\beta_{1–42}$ mediated deficit in LTP. We found that the neuroprotective effects of CBD were not reversed by WAY100635, ZM241385 or AM251, demonstrating a lack of involvement of $5HT_{1A}$, adenosine (A_{2A}) or Cannabinoid type 1 (CB_1) receptors respectively. However in the presence of the PPARγ antagonist GW9662 the neuroprotective effect of CBD was prevented. Our data suggests that this major component of *Cannabis sativa*, which lacks psychoactivity may have therapeutic potential for the treatment of AD.

Keywords Cannabidiol · Alzheimer's disease · Long-term potentiation · PPARγ · Beta amyloid peptide · $5HT_{1A}$ · Adenosine A_{2A} · CB_1R

Introduction

Alzheimer's disease (AD) causes a devastating decline in cognitive ability, leading to severe memory loss and confusion. The incidence of AD is increasing due to improved longevity, causing a major socio-economic burden. The post mortem pathological hallmarks of AD are the deposition of extracellular beta amyloid peptide (Aβ) plaques and intracellular neurofibrillary tangles comprised of hyper-phosphorylated tau protein. The amyloid cascade hypothesis (ACH) proposed that amyloid beta (Aβ) is the primary pathological trigger in AD [1]. Increased production, and/or decreased clearance of Aβ promotes the formation of toxic assemblies and ultimately plaques. Mutations in genes coding for amyloid precursor protein (APP) and presenilin 1 and 2 (PSEN1 and PSEN2) are present in familial cases of early onset AD, resulting in increased production of $A\beta_{42}$. Increased risk of sporadic AD is also linked to the APOE4 allele, associated with decreased Aβ clearance [2]. More recently, research has focussed on soluble forms of Aβ. Soluble extracts from human post-mortem brain tissue and cerebrospinal fluid (CSF), reveal variable proportions of Aβ oligomeric species, which correlate well with the degree of dementia [3–6].

Hippocampal long term potentiation (LTP), is an activity dependent form of synaptic plasticity, used routinely as a cellular model of memory formation [7] to assess potential novel therapies for AD [8, 9]. Soluble, oligomeric forms of synthetic $A\beta_{1–42}$ produce neuronal dysfunction, disrupting synaptic plasticity and hippocampal LTP [10–13]. Present AD therapies unfortunately, do not reliably reduce AD progression and do little to alleviate cognitive impairment. We have therefore investigated the potential neuroprotective effects of cannabidiol (CBD) a major non-psychoactive component of *Cannabis sativa*. CBD has known antioxidant, anti-inflammatory and neuroprotective effects [14–16]. In a mouse model of AD, CBD has been shown to improve social and object recognition memory [13]. It can also decrease Aβ-mediated cell death and neurotoxicity [14]. In addition, CBD can promote microglial migration, involved in

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Published online: 24 March 2018

the removal of Aβ [17] as well as increasing hippocampal neurogenesis and decreasing tau phosphorylation [18, 19]. CBD is known to act as a non-competitive negative allosteric modulator of CB_1 receptors [20], however it may also boost activity of the endogenous cannabinoid anandamide (AEA) by reducing uptake. There are many proposed mechanisms as to how CBD exerts its positive anti-inflammatory effects [21].

In our study, we have used an in vitro hippocampal slice model to investigate the effects of CBD on AD related neuronal dysfunction. We amongst others have previously reported an impairment of LTP following application of soluble amyloid derived diffusible ligands (ADDLs; $A\beta_{1-42}$) [10–12]. Here we have examined the effect of CBD on $A\beta_{1-42}$-mediated attenuation of LTP. CBD has previously been shown to alter synaptic transmission via activation of $5HT_{1A}$ [22]. In addition antagonists of the 5-HT_{1A} receptor have been shown to block CBD-mediated anxiolytic effects in animal models of anxiety, depression and stress [23–25]. CBD has also been shown to reverse the production of pro-inflammatory cytokines and chemokines via the adenosine A_{2A} receptor [26, 27]. Other targets of CBD include the nuclear PPARγ receptor, competition binding assay (IC50 5 μM), resulting in the induction of PPARγ mediated transcriptional activity [28]. CBD has been reported to lack affinity at the cannabinoid type 1 (CB_1) and CB_2 receptors [29], while other investigations have shown CBD to act as a weak antagonist at CB_1 and CB_2 [30]. Recently however CBD has been shown to act as a negative allosteric modulator of the endocannabinoid 2-AG as well as delta-9-THC [20]. CBD can also inhibit the uptake and metabolism of the endogenous cannabinoid anandamide [31], to potentially increase endocannabinoid tone.

In our study, we have investigated the neuroprotective effects of CBD against $A\beta_{1-42}$ and in addition have examined the potential role played by CBD at the $5HT_{1A}$ [22], adenosine A_{2A} [32] the cannabinoid type one (CB_1) receptor and the PPARγ receptor [28]. To elucidate the mechanism of action of CBD we examined the effect of combining CBD with receptor antagonists.

Materials and Methods

Animals

Hippocampal slices were prepared from 8 to 10 week old c57/black 6 mice (c57BL6), obtained from Charles River UK. Mice were housed in the biomedical facility, University College Dublin with a 12 h light/dark cycle with food and water ad libitum. All experiments were conducted under licence from the Dept of Health, Ireland (86/609/EEC).

Hippocampal Slices

Parasagittal hippocampal slices, 400-μm thick were prepared using a vibrotome (Leica VT1000S) as described previously [8] using ice cold cutting solution (mM) NaCl 87; $NaHCO_3$ 25; Glucose 25; Sucrose 75; KCl 2.5; NaH_2PO_4 1.25; $CaCl_2$ 0.5; $MgSO_4$ 7; bubbled with 95% O_2/5% CO_2 (carbogen). Slices were transferred to a holding chamber containing aCSF for recording (mM) NaCl 119; $NaHCO_3$ 26.2; Glucose 11 mM; KCl 2.5; NaH_2PO_4 1; $CaCl_2$ 2.5; $MgSO_4$ 1; bubbled with carbogen and were allowed to recover for at least 90 min at room temperature. Slices were transferred to a recording chamber, secured by means of a harp with fine nylon strings and perfused with recording aCSF at a rate of 5 ml/min maintained at 28–30 °C for the duration of all experiments. Recording electrodes were pulled from borosilicate capillary glass (GC150 F-10, Havard apparatus), using a horizontal puller (DMZ universal puller, Germany). Electrodes (2–5 MΩ) were filled with recording aCSF. The Shaffer-collateral pathway was stimulated using a monopolar electrode (FHC, Bowdoin, USA) at 0.033 Hz (duration: 100 μs), the return electrode was a silver/silver chloride wire placed in the recording bath. Extracellular field excitatory post synaptic potentials (fEPSPs) were recorded in the CA_1 *stratum radiatum* and paired stimuli were delivered with an inter-stimulus interval of 50 ms in order to monitor paired pulse facilitation (PPF). The voltage signal was filtered at 5 kHz and stored for off-line analysis using a personal computer interfaced with a CED/National Instruments A/D board and WinCP software (J. Dempster, Strathclyde University), or a Digidata 1440 and pclamp 10. Signals were amplified by a HS2A headstage (Molecular Devices, USA) connected to an Axoclamp 2B system (Molecular Devices, USA) and a Brownlee 410 Precision preamplifier. The stimulus voltage was adjusted to evoke a fEPSP that was 40–50% of the maximal response (maximum fEPSP just prior to the formation of a spike caused by cell firing) for the duration of all experiments. When a stable baseline had been recorded for 20 min a Master 8 (AMPI) timer was used to deliver two trains of high frequency stimuli (HFS), 100 Hz for 1 s, with an inter-train interval of 30 s. Following the application of HFS, the synaptic response was recorded for a further period of 60 min. All results are presented as mean ± SEM. The "*n*" numbers quoted refer to the number of slices used. Control and test experiments in a given section were conducted on the same day on slices from the same animal.

Preparation of Amyloid Derived Diffusible Ligands (ADDLs)

Synthetic human $A\beta_{1-42}$ (80% pure), was synthesized and purified using reverse phase HPLC by Dr. James Elliot, at the ERI amyloid laboratory (Oxford, CT, USA). $A\beta_{1-42}$ was dissolved in ice-cold HFIP (1,1,1,3,3,3-hexafluoro-2-propanol

[Sigma-Aldrich]), sonicated in an ultrasonic bath sonicator for 10 min and incubated at room temperature for 1 h in glass HPLC tubes. The HFIP was then evaporated using a gentle stream of N_2, and the remaining film was dissolved in anhydrous dimethylsulphoxide (DMSO) by vigorous vortexing. The solution was diluted in SILAC Advanced DMEM/F12 Flex Media (A2494301, Bio-Sciences), vortexed and incubated for 16 h at room temperature. Samples were centrifuged at 14,200 g for 15 min. The supernatant was removed, snap frozen in liquid N_2 and stored at − 80 °C, the pellet was disregarded.

Pharmacological Agents

CBD (STI Pharmaceuticals), WAY100635, AM251, ZM241385 and GW9662 (Tocris) were dissolved in 99+% dimethyl sulfoxide (DMSO Alfa Aesar) to a stock concentration and stored protected from light at − 20 °C. All drugs were added to the perfusion medium through a 40 ml reservoir. When testing antagonists, they were applied for 30 min, CBD was then applied for an additional 30 min followed by $A\beta_{1-42}$ for an additional 30 min before LTP was induced.

Drug Application and LTP

Following a 15–20 min stable baseline, receptor antagonist/CBD/ADDLs were added to the perfusate for 30 min and were present for the remainder of the experiment. In experiments examining a protective role for CBD on Aβ-ADDL mediated depression of LTP, CBD was added to the perfusate 30 min prior to the addition of Aβ, and was present for the remainder of the experiment. In experiments examining the mechanism of action of CBD, antagonists were added to the perfusate 30 min prior to CBD; ADDLs were added following a further 30 min prior to induction of LTP. HFS (100 Hz) was applied 30/60/90 min respectively following bath application of the combinations of test agents. Following HFS, the fEPSP amplitude was monitored for a further 60 min.

Data Analysis

The fEPSP amplitude was normalized to the average amplitude recorded for 10 min immediately prior to drug application or LTP induction. LTP was measured as the average increase in fEPSP amplitude recorded 55–60 min following high frequency simulation (HFS). PTP was measured over the 3 min period following HFS. Graphs were compiled and data analysed using Graphpad Prism. Results are expressed as mean ± SEM and were analyzed with ANOVA or students t-test, followed by Bonferroni's multiple comparison post hoc test. For all experiments comparing the effect of inhibitor + CBD + Aβ to the effects of Aβ + CBD + Aβ, the same data sets measuring the control, Aβ and CBD + Aβ-mediated effects on LTP are used for comparison; such groups are composed of pooled data sampled throughout the study.

Results

Acute Application of Cannabidiol (CBD) Increases Baseline Synaptic Transmission and Attenuates Aβ-ADDL-Mediated Deficits in LTP

A 30 min application of CBD (10 μM), caused a small increase in the fEPSP amplitude accompanied by a decrease in PPF (Fig. 1A, B). LTP induced in the presence of CBD (when baseline was normalized to 100% prior to LTP induction) was similar to control levels (Fig. 1C). PTP induced in the presence of CBD was also similar to control levels ($p > 0.05$) (Fig. 1D). When slices were perfused with βADDLs ($A\beta_{1-42}$; 500 nM) for 30 min prior to LTP induction, LTP (measured at 55–60 min post HFS) was significantly reduced compared to control (Fig. 2A, C). Post-tetanic potentiation (PTP) and PPF were similar in the control and Aβ-treated groups (Fig. 2D, E). Decreased levels of LTP in the presence of $A\beta_{1-42}$ are therefore likely to be independent of altered neurotransmitter release. When CBD was applied to slices 30 min prior to Aβ, the level of LTP induced was similar to control (Fig. 2B, $p > 0.05$). PTP and PPF were also unaltered by the combination of CBD + Aβ compared to either the control or Aβ treated group (Fig. 2D, E).

CBD Rescues Aβ-Mediated Deficits in LTP Through a Mechanism Independent of the $5HT_{1A}$, Adenosine A_{2A} or the CB_1 Receptor

As CBD at concentrations of 10 μM has previously been shown to act as an agonist at the $5HT_{1A}$ receptor [22], we investigated the effect of a potent $5HT_{1A}$ receptor antagonist WAY100635 on LTP and the protective effects of CBD. WAY100635 is known to bind $5HT_{1A}$ with high affinity (in vitro Kd 0.1 to 0.4 nmol/l) [33]. Application of WAY100635, (300 nM) did not alter baseline transmission significantly (99.3 ± 0.8, $n = 15$ at 30 min) or PPF (1.71 ± 0.04, $n = 15$). There was a small reduction in LTP in the presence of WAY 100635 compared to the control group ($p \leq 0.05$) (Fig. 3A), however PTP was unaltered. To determine if CBD was acting via the $5HT_{1A}$ receptor, WAY100635 was applied prior to CBD and Aβ. The CBD-mediated increase baseline EPSP amplitude was still present in the presence of WAY100635 (109 ± 8). LTP in the presence of WAY100635 + CBD + Aβ was not significantly different from LTP induced in the presence of CBD + Aβ (Fig. 4A, B) demonstrating the protective effects of CBD are not mediated via $5HT_{1A}$. PTP was also not significantly different between groups (Fig. 4E).

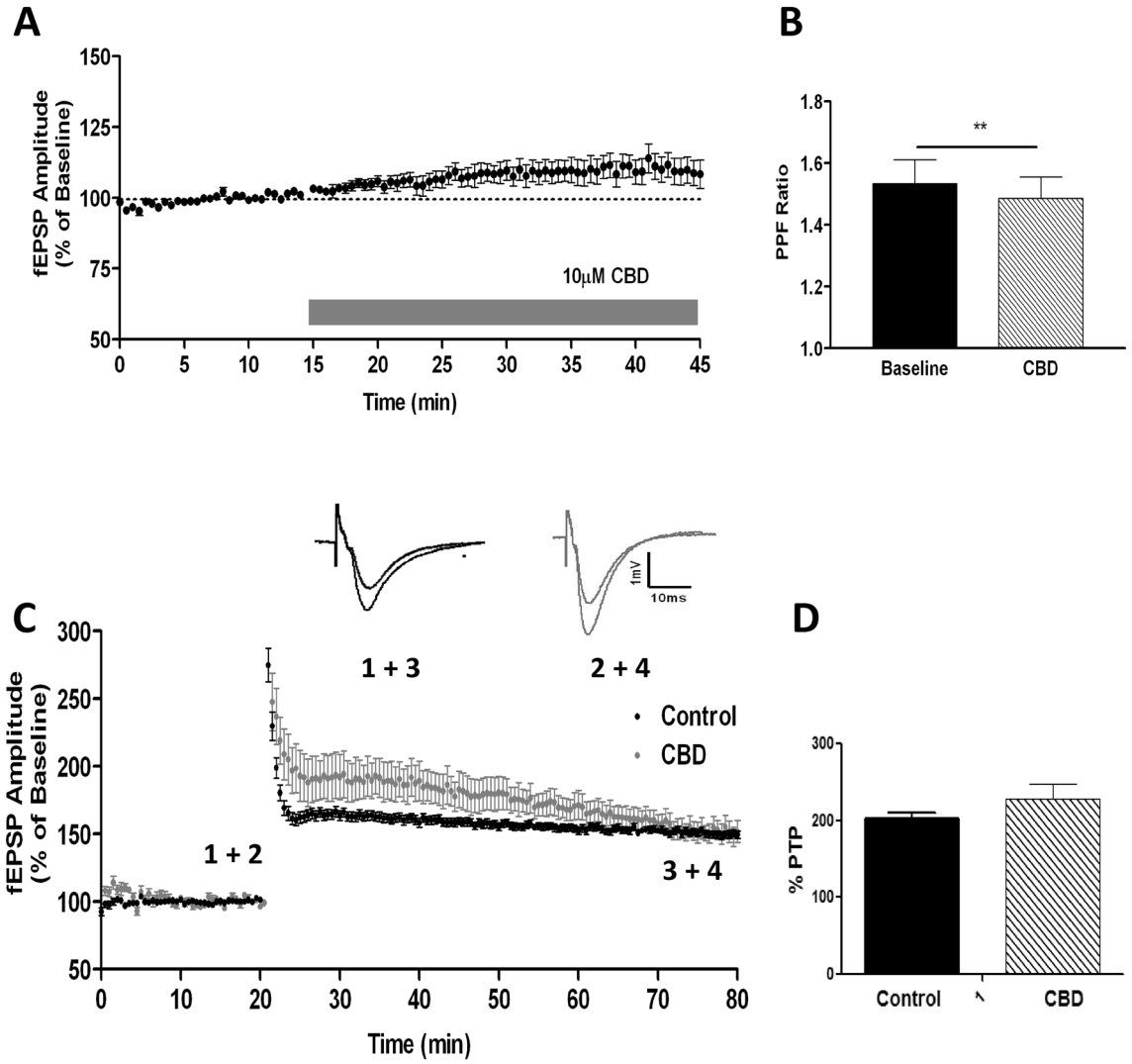

Fig. 1 **A** Baseline fEPSP amplitude was increased following a 30 min application of CBD (10 μM) (110.6 ± 4.1%; n = 16) compared to the normalized baseline (100.8 ± 0.4%; n = 16, $p < 0.05$). **B** Paired pulse facilitation ratio (1.53 ± 0.07; n = 14) is depressed following 30 min application of CBD (1.48 ± 0.07; n = 14). **C** LTP in the presence of CBD (152.3 ± 7.7%; n = 11) was similar to control (148.6 ± 2.4%; n = 58, $p > 0.05$). **D** PTP in the presence CBD (228 ± 19; n = 11) was similar to control (203 ± 8). Sample traces represent fEPSPs recorded before and 60 min following HFS from control (1 + 3) and CBD (2 + 4) treated slices

CBD has also been shown to disrupt adenosine reuptake and subsequently, to enhance activation of the adenosine A_{2A} receptor [32]. LPS induced production of proinflammatory cytokines is also reversed by CBD in an A_{2A}-dependent manner [27]. We therefore examined a role for the A_{2A} receptor in regulating the neuroprotective effects of CBD on the Aβ-mediated impairment of LTP. ZM241385 is a high affinity A_{2A} antagonist, shown to be neuroprotective at concentrations of 50 nM [34]. Application of the A_{2A} receptor antagonist, ZM241385 (100 nM) did not significantly increase baseline synaptic transmission (108 ± 4, n = 14, at 30 min) or alter PPF (1.64 ± 0.06, n = 14). LTP was however slightly but significantly reduced in the presence of ZM241385 compared to the control group ($p < 0.05$; Fig. 3B) while PTP was unaltered. Application of ZM241385 prior to CBD and Aβ did not alter the neuroprotective effects of CBD as LTP recorded in slices in the presence of ZM + CBD + Aβ was significantly enhanced compared to slices treated with Aβ alone and similar to ZM alone (Fig. 4B, D). PPF was similar in ZM + CBD + Aβ (1.6 ± 0.1, n = 9), and CBD + Aβ (1.6 ± 0.06, n = 8) treated slices as was PTP (Fig. 4E).

Given its known role in modulating CB_1 receptor activity [20] and the fact it can indirectly activate CB_1 via reduced uptake of anandamide (AEA) we investigated a possible interaction with the CB_1 receptor using the CB_1 inverse agonist AM251 (2 μM). Application of AM251 to hippocampal slices caused a small increase in baseline synaptic transmission which did not reach statistical

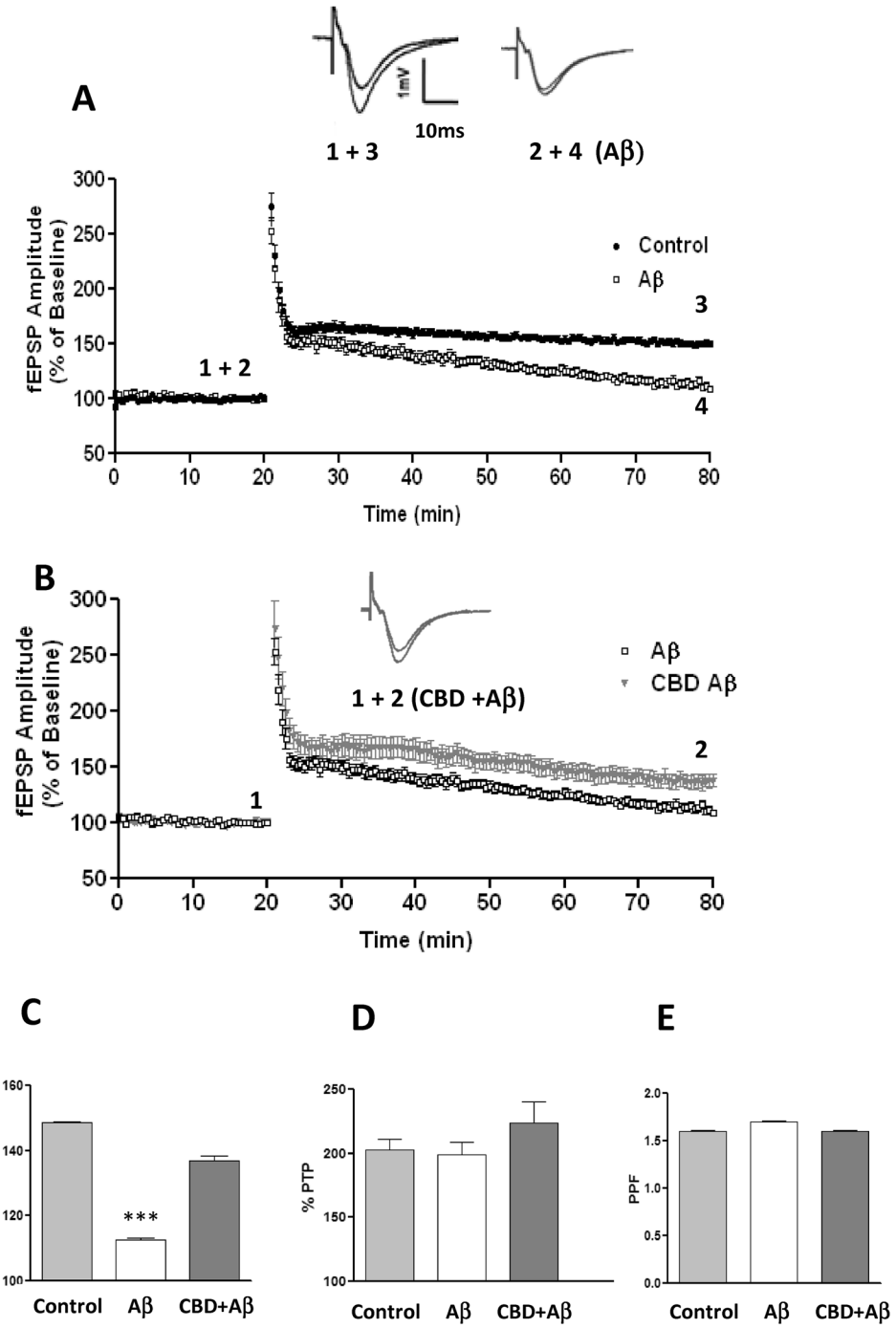

Fig. 2 Treatment of slices with CBD attenuated the Aβ-mediated deficit in LTP. LTP was significantly attenuated in the presence of $A\beta_{1-42}$ (500 nM) (112.6 ± 2.9%; n = 23) compared to control (148.6 ± 2.4%; n = 59; **A** and **C**). **B** Application of CBD to slices 30 min prior to addition of Aβ attenuated the Aβ-mediated deficit in LTP (136.9 ± 5.9%; n = 15, $p \leq 0.001$) compared to Aβ alone. LTP in the presence of CBD and Aβ was similar to control ($p > 0.05$). **D** PTP was similar in control (203 ± 8%, n = 58), Aβ (198.9 ± 9.8%; n = 23) and CBD + Aβ; (224 ± 16.5%; n = 15) treated groups. **E** PPF ratio (60 min post HFS) was also similar in control (1.6 ± 0.04; n = 58), Aβ (1.7 ± 0.05; n = 23) and CBD + Aβ (1.6 ± 0.06) treated groups

significance (115.5 ± 8.6, n = 15). Although LTP evoked in the presence of AM251 was also slightly elevated, this was also not significantly different to control (Fig. 3C). PTP and PPF in AM251 (Fig. 3C) were also similar to the control group. LTP in the presence of AM251 + CBD + Aβ was similar to levels in CBD + Aβ (Fig. 4C, D). PTP (Fig. 4E) and PPF measured in AM 251 + CBD + Aβ were similar to control demonstrating that AM251 did not alter the protective effects of CBD on restoring LTP in slices treated with $A\beta_{1-42}$. CBD therefore appears to act via a CB_1 receptor independent mechanism.

The PPARγ Antagonist GW 9662 Does Not Alter LTP but Attenuates the Neuroprotective Effects of CBD

It has previously been reported that CBD can act as a PPARγ agonist [28] and can inhibit Aβ-mediated pro-inflammatory activity via PPARγ [19], we therefore examined the effects

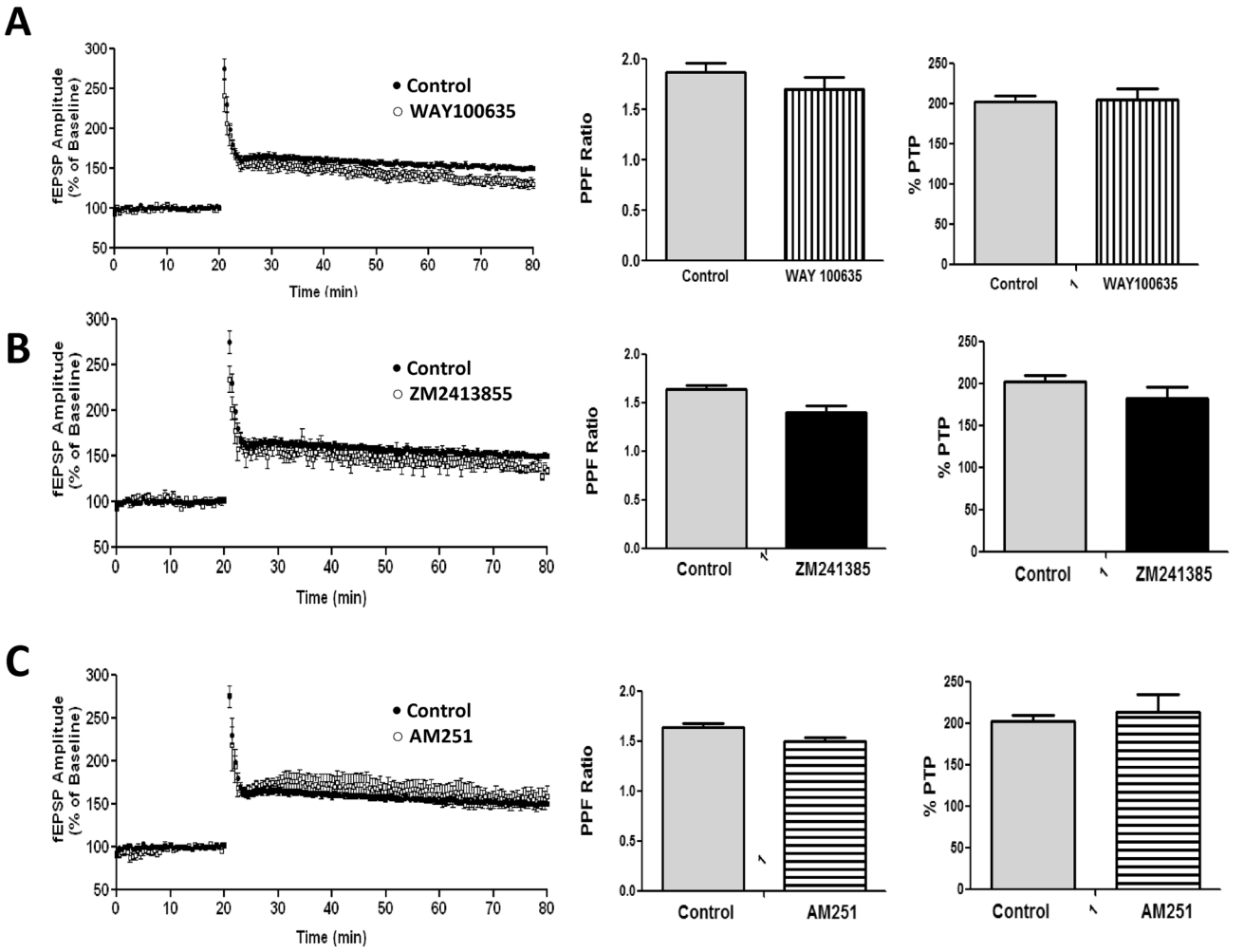

Fig. 3 Effects of $5HT_{1A}$, Adenosine A_{2A} and CB_1 receptor antagonists on LTP, PTP and PPF. **A** LTP was reduced in the presence of WAY100635 (135.1 ± 4.3%; n = 8) compared to control, ($p < 0.05$), PTP (205.6 ± 13%; n = 8) and the PPF ratio were similar to control. **B** The A_{2A} receptor antagonist, ZM241385 caused a small reduction in LTP (130.2 ± 5.3%; n = 9) compared to control ($p < 0.05$). PTP (183.0 ± 13.7%; n = 9) and PPF (1.4 ± 0.07) were similar to control. **C** LTP in AM251 (156.0 ± 11.2%; n = 7) was similar to control as were PTP (214.8 ± 21; n = 7) and PPF (1.5 ± 0.04)

of the antagonist GW9662 (2 μM). GW9662 is known to have an IC50 in the nM range versus PPARγ and to bind irreversibly to the receptor [35]. The CBD-mediated increase in fEPSP amplitude (110.6 ± 4.1%; n = 16) was unchanged in the presence of GW9662 (119.4 ± 11.1%). LTP and PTP induced in the presence of GW9662 were also similar to control (Fig. 5A). We investigated a potential role for PPARγ in the CBD mediated neuroprotective effect against Aβ. Prior treatment with GW9662 reduced the neuroprotective effects of CBD. Levels of LTP in the presence of GW + CBD + Aβ were significantly lower than those recorded in the presence of CBD and Aβ ($p < 0.05$) and were similar to those recorded following treatment with Aβ alone (Fig. 5B). Reversal of the effects of CBD by GW9662 was independent of any change in PTP or PPF which were similar to levels recorded in the presence of CBD + Aβ and Aβ alone (Fig. 5C, D).

Discussion

Here we have shown for the first time that prior treatment of hippocampal slices with CBD can attenuate the effects of $A\beta_{1-42}$ on LTP in the CA_1 region. In our experiments investigating the acute effects of Aβ and CBD on LTP, prior treatment with the $5HT_{1A}$, A_{2A} or CB_1 receptor antagonists failed to reduce the restorative effects of CBD. The protective effect of CBD was however reversed in acute slices following prior treatment with a PPARγ antagonist. Application of CBD to slices enhanced baseline synaptic transmission which was accompanied by a decrease in PPF suggesting an increase in neurotransmitter release. This could be accounted for by a CBD mediated subtle increase in intracellular $[Ca^{2+}]$, via Ca^{2+} release from intracellular stores and also from mitochondria [36]. This increase in $[Ca^{2+}]$ may

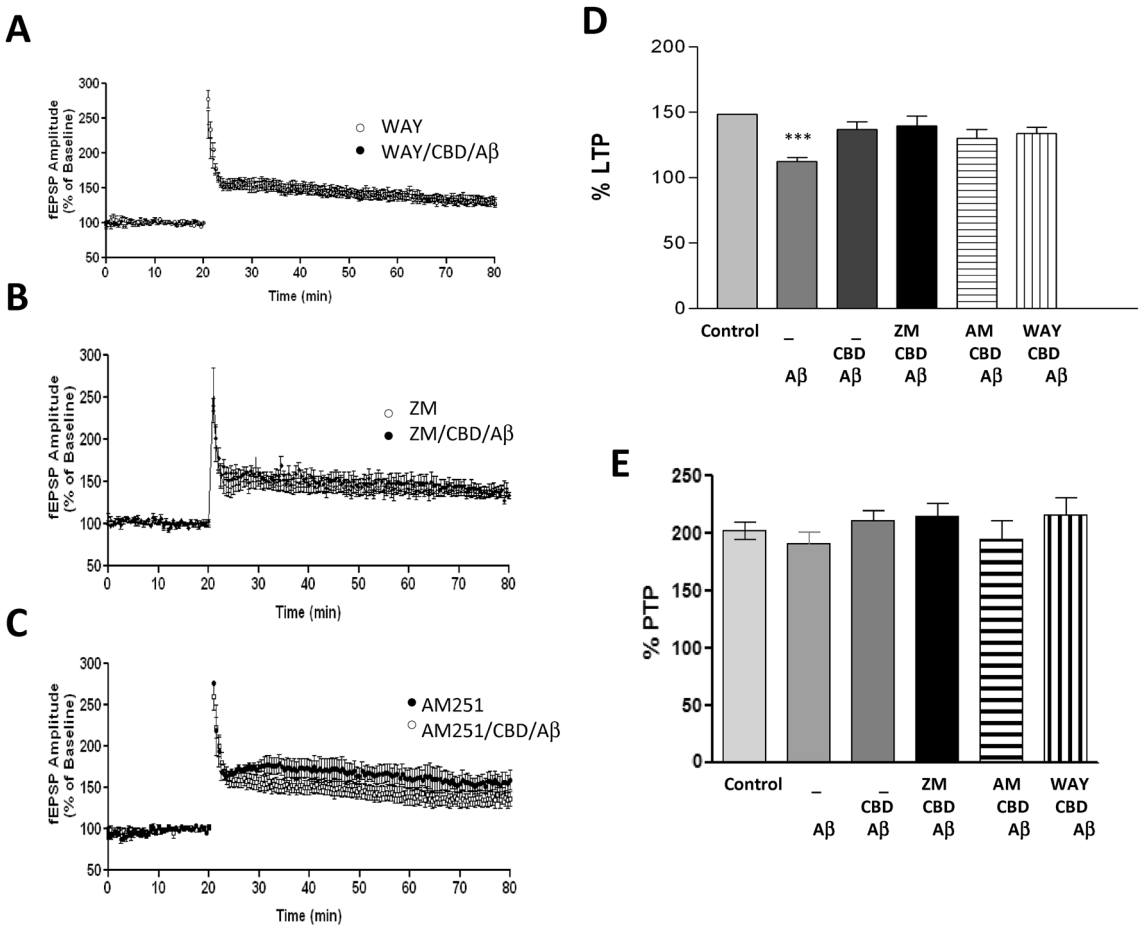

Fig. 4 CBD rescues Aβ-mediated deficits in LTP through a mechanism independent of the. $5HT_{1A}$, Adenosine A_{2A} and CB_1 receptor. **A** LTP in the presence of WAY100635 open circle was similar to WAY + CBD + Aβ filled circle, (131 ± 5.2%; n = 14 p > 0.05). The PPF ratio was unaltered by WAY + CBD + Aβ (1.6 ± 0.04) compared to WAY alone. **B** LTP in the presence of ZM241385 filled circle was similar to ZM241385 + CBD + Aβ filled circle (139.6 ± 7.3%; n = 9, p > 0.05) and CBD + Aβ. PPF was also similar in ZM and ZM + CBD + Aβ (1.6 ± 0.1) treated slices. **C** LTP in AM251 filled circle and in the presence of AM251 + CBD + Aβ open circle (133.1 ± 6.6%; n = 13) are not significantly different. PPF (1.6 ± 0.05; n = 14, p > 0.05) remained similar to control, Aβ alone and CBD + Aβ groups (p > 0.05). **D** Summary of LTP. **E** PTP summary in control, Aβ alone and in the presence of CBD, Aβ and ZM/WAY/AM251

then cause the small but sustained increase in basal transmitter release. It was also possible to induce robust LTP in the presence of CBD suggesting that this agent is unlikely to have any inhibitory effect on learning as shown in behavioural models [13].

In agreement with previous studies, a 30 min application of $A\beta_{1-42}$ (500 nM) to hippocampal slices resulted in inhibition of LTP in the CA_1 region [12]. Other groups, using oligomeric Aβ, have also reported no decay in basal synaptic transmission, PPF or PTP following acute incubation with sub-micromolar concentration of Aβ [37]. In an effort to determine the mechanism of action of CBD we examined the effects of the $5HT_{1A}$ receptor antagonist WAY100635. This agent has previously been shown to have no effect on excitability or EPSP amplitude in the CA_1 region [38]. A previous report demonstrated that CBD could depress basal synaptic transmission in rat hippocampal slices [22] via a $5HT_{1A}$ and CB_1 dependent mechanism. This effect was possibly caused by a CBD mediated decrease in reuptake of anandamide (AEA), producing indirect activation of CB_1 receptors [39]. In our mouse hippocampal slices however, activation of presynaptic CB_1 by AEA may have been occluded by higher levels of endogenous adenosine (eADO). It has been shown that CB_1 and adenosine A1 receptor (A_1R) share a common presynaptic signalling mechanism at glutamatergic synapses. As eADO levels are higher in mouse than in rat slices, A_1R control of the common CB_1 linked G-protein is increased, masking the effects of CB_1 activation [40]. In addition, recent research demonstrating that CBD is a negative allosteric modulator at CB_1 [20], could also account for the lack of indirect agonist effects at this receptor. CBD has also been shown to act as an antagonist at CB_1 and CB_2R [41] and recently to act as a negative allosteric modulator at CB_2R [42].

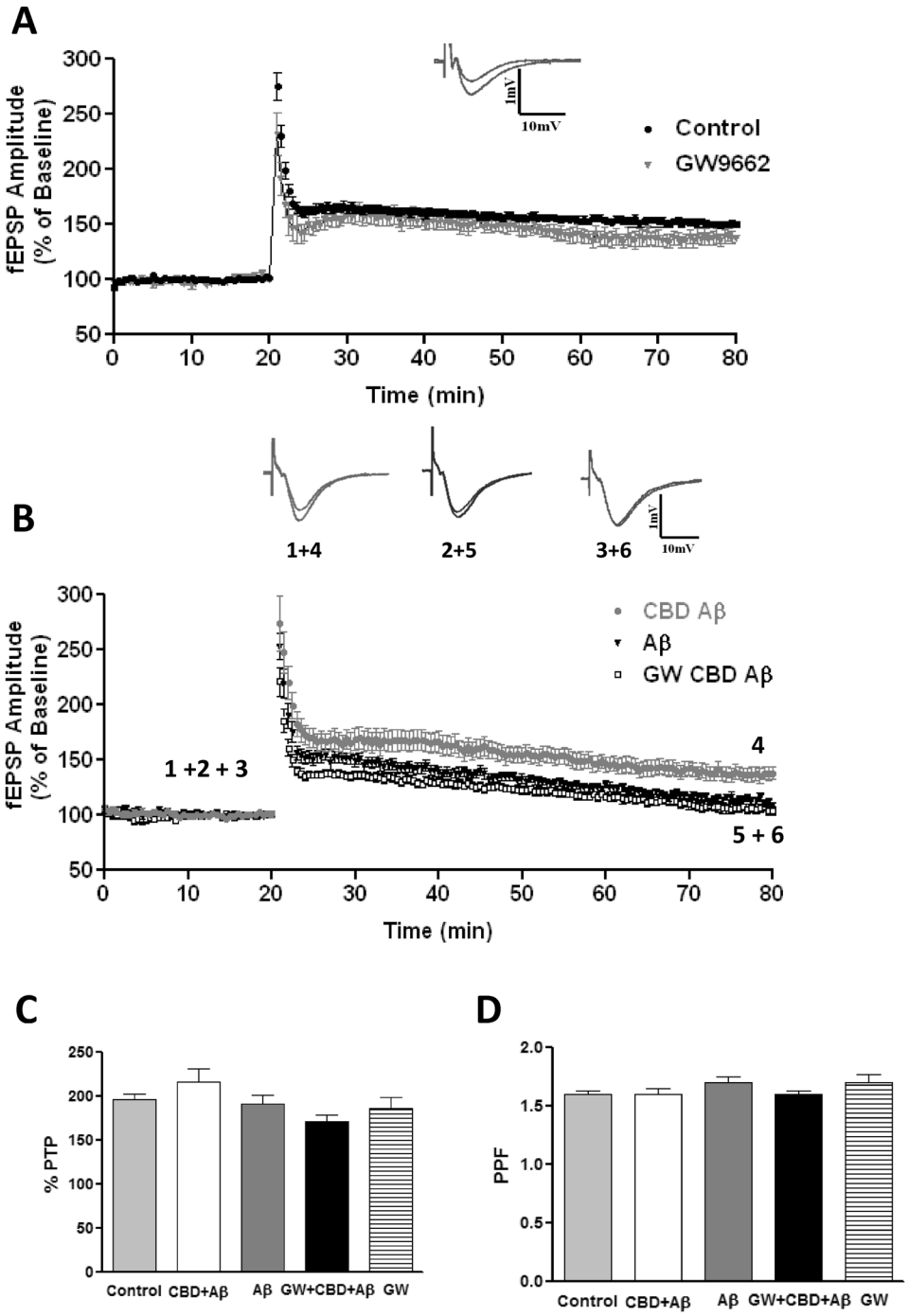

Fig. 5 The PPARγ antagonist GW 9662 did not significantly reduce LTP but attenuated the neuroprotective effect of CBD. **A** Data trace shows LTP in a GW treated slice. LTP in the presence of GW 9662 (137.7 ± 5.7%; n = 7) was similar to control. **B** Data traces were recorded at the times indicated by numbers 1–6. Application of GW9662 prior to CBD reduced the neuroprotective effects of CBD against Aβ. Levels of LTP in GW + CBD + Aβ (106.8 ± 4.2%; n = 9 $p \leq 0.001$) were significantly lower than those recorded in CBD + Aβ ($p < 0.01$) and were similar to those recorded in Aβ alone. PTP and PPF (**C**, **D**) were not significantly different from control (mean ± SEM)

Application of the A_{2A} receptor antagonist ZM241385 to hippocampal slices did not alter basal synaptic transmission or PPF but it did depress LTP to a small extent, in contrast to a previous study [43]. While A_{2A} receptors have a low density in the hippocampus, A_{2A} agonists have been shown to enhance output from CA_1 cells [44]. This latter finding may explain the small depression in LTP which we observed. Pre-treatment of slices with ZM241385 prior to CBD and Aβ application did not however alter the protective effect of CBD suggesting that this receptor is not involved. There is evidence that CBD can inhibit adenosine reuptake [45], and via indirect activation of the A_{2A} receptor, it can reduce levels of TNFα and IL1β [26, 27, 32]. The indirect activation of A_{2A}Rs by CBD also exerts neuroprotection *via* down regulation of caspase 9, and reversal of glutamate induced toxicity [46]. CBD mediated indirect activation of this receptor would appear to be beneficial in neurodegenerative conditions,

it is however not involved in the acute effects we have observed here.

In agreement with a previous study, antagonism of the CB_1 receptor did not affect LTP elicited with robust (100 Hz) stimulation [47]. Modulation of the eCB system has been implicated in AD, Levels of diacylglycerol lipase α (DAGL-α) are upregulated and its hydrolysing enzyme monoacylglycerol lipase MAGL down regulated in post-mortem AD brains resulting in the enhanced levels of 2-AG [48]. Aβ-mediated impairments in an inhibitory avoidance task are reversed by the CB_1 receptor antagonist SR141716A, suggesting a role for CB_1 receptors [49]. In contrast, we did not observe a protective effect of CB_1 receptor blockade by AM251 against Aβ (data not shown). AM251 also failed to reverse the acute neuroprotective effects of CBD suggesting a lack of effect at the receptor or of endogenous cannabinoids.

We found that the PPARγ antagonist GW9662 (2 μM) had no effect on LTP in agreement with an earlier observation [9] The PPARγ antagonist however reversed the effects of CBD in our study suggesting that activation of this nuclear receptor is central to the effects of CBD. PPARγ is a nuclear receptor expressed at low levels in the CNS with increased expression under inflammatory conditions. Activation of PPARγ inhibits pro-inflammatory gene expression through the inhibition of NFkB [50]. Moreover, PPARγ is reported to promote macrophage differentiation towards the M2 anti-inflammatory phenotype and to repress the pro-inflammatory M1 phenotype [51]; a beneficial effect regarding possible longer term treatment with CBD or other PPARγ agonists. CBD has been shown to bind to PPARγ resulting in the induction PPARγ-mediated transcriptional activity [28]. Further, CBD inhibits Aβ-mediated expression of the NFkB p50/p65 complex, inhibiting pro-inflammatory NFkB transcriptional activity in a PPARγ-dependent manner [19]. This inhibition of NFkB contributed to a CBD induced reduction in iNOS, GFAP, IL1β, TNFα and S100B in Aβ injected rats [19]. PPARγ agonists have also been shown to reverse Aβ-mediated depression of LTP in acute rat hippocampal slices [9]. As the latter experiments were conducted using $Aβ_{1–40}$, it would be interesting to examine the effects of troglitizone, ciglitizone and PGJ2 against $Aβ_{1–42}$ ADDLs used in our present study.

Considering the neuroprotective effect of CBD and how it is reduced by PPARγ antagonist GW9662, there are many signalling cascades that are likely to be involved. Inhibition of LTP following acute application of Aβ has also been shown to involve activation of caspase-3, cleavage of Akt and subsequent increased activation of glycogen synthase kinase 3β (GSK3β) [52]. Aβ-mediated activation of GSK3β can inhibit early LTP an effect that is reversed by pharmacological inhibition of caspase-3 or GSK3β or via expression of an Akt mutant which is resistant to caspase-3 [52]. Activation of GSK3β is known to favour long term depression (LTD) as opposed to LTP [53]. Increased caspase-3 activity either produced by Aβ or by brain-iron loading can also be reversed by CBD [14, 54], the latter effect is associated with improved mitochondrial function. In the iron loading model CBD reversed memory deficits in the novel object recognition test and in an inhibitory avoidance task, possibly due to CBD mediated down regulation of caspase-3 [55].

The transcriptional activator β-catenin controls the expression of anti-inflammatory genes and is thought to be a target for PPARγ. β-catenin is inhibited by Aβ and can be reversed by CBD suggesting a modulatory role for CBD in the canonical Wnt signalling pathway [56]. Agonists of PPARγ are thought to have anti-inflammatory action by inhibiting NF-kB mediated transcription of gene targets [57]. Stimulation of PPARγ decreases GSK3β activity [58] which should also facilitate LTP. An interaction and cross talk between CBD, PPARγ and the Wnt/β-catenin pathway is likely to play a major role in the neuroprotective effects of CBD [21].

Considering the mounting evidence that CBD can reduce neuroinflammation and protect against the effects of Aβ both in vitro and in some in vivo models [13] this non psychoactive cannabinoid should be given serious consideration as a possible novel therapy for the treatment of AD.

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