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The direct actions of cannabidiol and 2-arachidonoyl glycerol at $GABA_A$ receptors

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ABSTRACT

Cannabidiol (CBD) is a major non-intoxicating component of cannabis and possesses anti-epileptic, anxiolytic and anti-hyperalgesic properties. The mechanism of action of CBD in producing such effects remains unclear. Despite evidence that some endogenous and synthetic cannabinoids interact with $GABA_A$ receptors, no-one has yet investigated the effects of CBD. Here we used two-electrode voltage clamp electrophysiology to compare the actions of CBD with those of the major central endocannabinoid, 2-arachidonoyl glycerol (2-AG) on human recombinant $GABA_A$ receptors (synaptic $\alpha1\text{-}6\beta\gamma2$ and extrasynaptic $\alpha4\beta2\delta$) expressed on *Xenopus* oocytes. CBD and 2-AG were positive allosteric modulators at $\alpha1\text{-}6\beta\gamma2$ receptors, with low micromolar potencies. The maximal level of enhancement seen with either CBD or 2-AG were on $\alpha2$-containing $GABA_A$ receptor subtypes, with approximately a 4-fold enhancement of the GABA EC_5 evoked current, more than twice the potentiation seen with other α-subunit receptor combinations. Further we observed β-subunit selectivity, whereby modulatory activity was higher at $\beta2/\beta3$ over $\beta1$ subunits. The $\beta1$-subunit homologous mutant $\beta2(V436T)$ substantially diminished the efficacy of both drugs to a third of that obtained with wild-type $\beta2$ subunit combinations, but without changing potency. The potency of CBD increased and efficacy preserved in binary $\alpha1/\alpha2\beta2$ receptors indicating that their effects do not involve the classic benzodiazepine site. Exploration of extrasynaptic $\alpha4\beta2\delta$ receptors revealed that both compounds enhanced GABA EC_5 evoked currents at concentrations ranging from 0.01–1 μM. Taken together these results reveal a mode of action of CBD on specifically configured $GABA_A$ receptors that may be relevant to the anticonvulsant and anxiolytic effects of the compound.

1. Introduction

GABA mediates fast inhibitory neurotransmission in the mammalian central nervous system (CNS) via its actions on $GABA_A$ receptors. $GABA_A$ receptors form part of the Cys-loop receptor family of ion channels which include the nicotinic acetylcholine, serotonin 3 ($5\text{-}HT_3$) and strychnine-sensitive glycine gated receptors. $GABA_A$ receptors exhibit high receptor subtype heterogeneity, assembling from a combination of subunits derived from a family of 19 genes, and include $\alpha1\text{–}6$, $\beta1\text{–}3$, $\gamma1\text{–}3$, δ, π, ε, θ and $\rho1\text{–}3$ subunits [1,2]. These subunits co-assemble to form heteropentamers that allow the conductance of chloride ions through a central pore. The majority of $GABA_A$ receptors are composed of two α, two β and either one γ or a δ subunit [1,2].

$GABA_A$ receptors are the target of numerous clinically-relevant drugs such as benzodiazepines (BDZ), barbiturates and general anaesthetics [1,2], as well as natural products including kavalactones and flavonoids [3,4], with the specific subunit configuration determining the pharmacological properties of these receptors. Additionally, the brain distribution of these receptors is regionally specific and each combination mediates a distinct physiological role. For example, $GABA_A$ receptors containing $\alpha1\text{–}3$ subunits

Abbreviations: CBD, cannabidiol; 2-AG, 2-arachidonoyl glycerol; $GABA_A$ receptor, γ-amino butyric acid receptor type A; EC, effective concentration; CNS, central nervous system; 5-HT, $_{33}$serotonin 3; AEA, anandamide; CB1, cannabinoid receptor 1; CB2, cannabinoid receptor 2; THC, Δ9-tetrahydrocannabinol; DMSO, dimethyl sulphoxide; DS2, delta selective compound 2; BDZ, benzodiazepines.

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are localised in the synapse and mediate fast synaptic inhibition (termed phasic inhibition). In contrast, α4–6 subunit containing $GABA_A$ receptors are found largely extrasynaptically and mediate a form of tonic inhibition [5,6]. Most γ containing receptors are localised in the synapse whilst the δ subunit, co-assembles predominantly with α4 /α6 subunits to reside at perisynaptic or extrasynaptic sites [5,6]. Thus $GABA_A$ receptors play a major role in the maintenance of synchronised, well-orchestrated communications between relevant neural networks, and as such, changes in $GABA_A$ receptor neurotransmission have implications for neurological and psychiatric disorders including epilepsy, insomnia, anxiety, stroke and schizophrenia [7,8].

Distinct $GABA_A$ receptor subtypes have been implicated in these various disorders and may represent useful therapeutic targets. Indeed, preclinical studies using transgenic mice and/or subtype selective ligands support the attribution of receptor subunits to specific disorders and/or to the pharmacological actions of established therapeutics. These include: α1 receptor subtypes which are implicated in epilepsy and in the sedative/hypnotic effects of drugs; α2/α3 receptor subtypes which are involved in anxiolytic actions of drugs; and α5 receptor subtypes which are implicated in learning, memory and the action of nootropic drugs [9]. Further, different β-subunits mediate distinct functional associations, including β2 /β3 receptor subtypes being implicated in sedation, sleep, epilepsy, and anaesthesia [10].

Synthetic, plant-based and endogenous cannabinoids comprise diverse families of compounds, some of which exert their main effects via G-protein coupled CB1 and/or CB2 cannabinoid receptors [11]. CB1 receptors are ubiquitously expressed in the CNS and are involved in the presynaptic regulation of GABA and glutamate release [12]. 2-Arachidonoyl glycerol (2-AG) and anandamide (*N*-arachidonoyl ethanol amide, AEA) are the major endogenous lipid neurotransmitters (endocannabinoids) that are synthesised on demand and act upon presynaptic CB1 receptors via retrograde signalling mechanisms [13]. 2-AG is more abundant than AEA in the CNS, where it acts with higher affinity and intrinsic activity on both CB1 and CB2 receptors [14,15].

The phytocannabinoid, cannabidiol (CBD), is a major non-intoxicating component of cannabis and is a structural isomer of the main intoxicating compound Δ9-tetrahydrocannabinol (THC). Unlike THC, CBD displays low affinity for the orthosteric site of CB1 receptors and acts as a neutralising modulator with high affinity on an allosteric site [16,17]. Despite an apparent lack of overt psychoactive effects, CBD has a range of functional effects and can reduce anxiety, depression, pain, psychotic symptoms and seizures, including treatment-resistant pediatric epilepsies such as Dravet syndrome [11,18]. Dravet syndrome typically arises from a genetic mutation of the sodium channel Nav1.1. This channel is expressed predominantly on inhibitory GABAergic interneurons, such that the mutation leads to abnormal excitability and seizures [19]. Recent clinical trials indicate that CBD can have a profound effect in reducing seizure numbers in a substantial subset of Dravet sufferers [18,20]. However, no known mechanism of action accounts for the anticonvulsant effects, or for many of the other clinically relevant actions of CBD [18].

Recently, the endocannabinoids 2-AG and AEA were characterised as positive allosteric modulators of $GABA_A$ receptors, potentially accounting for some of their physiological effects [21,22]. However, no studies to date have evaluated the effects of CBD on $GABA_A$ receptors. CBD, THC, 2-AG and other fatty acid neurotransmitters have been found to act as allosteric modulators on a variety of Cys-loop gated receptors other than $GABA_A$ receptors, including glycine receptors, $5\text{-}HT_3$ receptors and α7 nicotinic acetylcholine receptors [23–33]. Accordingly, here we performed a full characterisation of both CBD and 2-AG on 10 human recombinant $GABA_A$ receptors expressed in *Xenopus* oocytes using two-electrode voltage clamp methods.

The data show that CBD and 2-AG were positive allosteric modulators at all α containing $GABA_A$ receptors but with a higher efficacy for the α2-containing receptor. CBD and 2-AG exhibited higher efficacy at α2β2γ2L and α2β3γ2L receptors compared to α2β1γ2L $GABA_A$ receptors, indicating preference for β2/β3 over β1-containing receptors. We then assessed whether the point mutation β2V436T found in the TM4 region affects the actions of either CBD or 2-AG on α2β2(V436T)γ2L receptors. Whilst the mutation attenuated the ability of these compounds to enhance GABA currents, the potencies were similar to that of wild-type α2β2γ2L receptors. Additionally, the modulatory actions of CBD and 2-AG were not mediated via the classical benzodiazepine α-γ2L interface. Finally, characterization of CBD and 2-AG mediated GABA enhancement on extrasynaptic, δ-containing receptors is reported. These actions of CBD on $GABA_A$ receptors may contribute to the anti-seizure and anxiolytic effects seen with this compound.

2. Materials and methods

2.1. Compounds

GABA, zinc chloride, delta selective 2 (DS2) and dimethyl sulphoxide (DMSO) were purchased from Sigma-Aldrich (Sydney, Australia). CBD was obtained from THC Pharm (Frankfurt, Germany) as the crystalline solid. 2-AG was obtained from Cayman Chemicals (Ann Arbor, MI, USA) as a 9:1 mixture of 2-AG and 1-AG (a spontaneous isomer) in acetonitrile. 2-AG and CBD were prepared as 100 mM stock solutions in DMSO prior to use. The final experimental solutions of CBD and 2-AG contained no more than 0.8% DMSO which when applied alone did not produce any alteration in electrophysiological recordings.

2.2. $GABA_A$ receptor subunit constructs

Human complementary DNA (cDNA) for α1, β2 and γ2L $GABA_A$ receptor subunits subcloned into pCDM8 vectors were provided by Dr Paul Whiting (Merck, Sharpe and Dohme Research Labs, Harlow, UK). cDNA for human α3, α4, α5, α6, β1, β3 and δ subunits (vectors: pGEMHE, pCDNA3, pCDM8. pCDNA1) were a gift from Dr Bjarke Ebert (H. Lundbeck A/S,Valby, Denmark). Human α2 cDNA subcloned into pCMV6-XL5 was purchased from OriGene Technologies (Rockville, MD, USA). The cloned DNA underwent complete sequencing to confirm the sequence (Australian Genome Research Facility, Westmead, NSW, Australia).

Each cDNA containing vector were linearised with restriction endonucleases as follows; α1, α5, α6, β1, β2, γ2L using NotI; α2 using SmaI; α3 and β3 using NheI; α4 and δ using HpaI. Capped RNA transcripts were synthesised from linearised plasmids using the mMessage mMachine T7 transcript kit (Ambion, Austin, TX, USA). Lithium chloride precipitated cRNA was diluted in nuclease-free water and stored at −20 °C. The quality of cRNA was determined by 0.45% agarose gel electrophoresis and mRNA concentrations were measured by NanoDrop® ND-1000 UV–vis Spectrophotometer.

2.3. Xenopus laevis *oocytes preparation*

Surgical procedures on *Xenopus laevis* were approved by the Animal Ethics Committee of the University of Sydney (reference number: 2013/5915). The procedure for the harvesting and enzymatic separation of *X. laevis* oocytes was identical to that described previously [34–36]. Briefly, oocytes were harvested by anaesthetising a sexually mature female *X. laevis* by immersion in 0.17% tricaine with 0.02% NaCl for 10–15 min. Immediately after and performed on ice, the anaesthetised animal had a lobe of the ovaries surgically