British Journal of Pharmacology

British Journal of Pharmacology (2017) •• ••–•• 1

RESEARCH PAPER

Antidote to cannabinoid intoxication: the CB_1 receptor inverse agonist, AM251, reverses hypothermic effects of the CB_1 receptor agonist, CB-13, in mice

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Received 17 May 2017; **Revised** 21 June 2017; **Accepted** 3 August 2017

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BACKGROUND AND PURPOSE

Cannabis is a recreational drug leading to intoxication, following stimulation of cannabinoid CB_1 receptors. However, more recently, herbs mixed with synthetic cannabinoids sometimes known as 'Spice' and 'Black Mamba' have been increasingly used, and their high CB_1 receptor affinity has led not only to marked intoxication but also life-threatening complications and an increasing number of deaths. Although many studies have indicated that prophylactic treatment with CB_1 receptor antagonists can block cannabimimetic effects in animals and humans, the aim of this study was to determine whether CB_1 receptor antagonism could reverse physical cannabimimetic effects.

EXPERIMENTAL APPROACH

Cannabimimetic effects, measured by the hypothermic response following sedation and hypomotility, were induced by the synthetic CB_1 receptor agonist CB-13 (1-naphthalenyl[4-(pentyloxy)-1-naphthalenyl]methanone) in Biozzi Antibody High mice. The CB_1 receptor antagonist/inverse agonist AM251 (*N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2, 4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide) was administered 20 min after the injection of CB-13 and its effects on the cannabimimetic responses were assessed.

KEY RESULTS

In this study, the CNS-related cannabimimetic effects, as measured by the hypothermic effect, induced by the CB_1 receptor agonist were therapeutically treated and were rapidly reversed by the CB_1 receptor antagonist/inverse agonist. There was also a subjective reversal of visually evident sedation.

CONCLUSIONS AND IMPLICATIONS

Cannabinoid receptor antagonists have been widely used and so may provide an acceptable single-dose antidote to cannabinoid intoxication. This use may save human life, where the life-threatening effects are mediated by cannabinoid receptors and not off-target influences of the synthetic cannabinoids or non-cannabinoids within the recreational drug mixture.

Abbreviations

AM251, N-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide; AM2201, 1-(5-fluoropentyl)-3-(1-naphthoyl) indole; CB-13, 1-naphthalenyl[4-(pentyloxy)-1-naphthalenyl]methanone; JWH-018, 1-pentyl-3-(1-naphthoyl)indole; JWH-122, 4-methyl-1-naphthalenyl)(1-pentyl-1*H*-indol-3-yl)methanone; JWH-250, 1-pentyl-3-(2-methoxyphenylacetyl)indole; JWH-251, 2-(2-methylphenyl)-1-(1-pentyl-1*H*-indol-3-yl)ethanone; THC, tetrahydrocannabinol

DOI:10.1111/bph.13973

Introduction

Cannabis sativa is a mind-altering recreational drug that contains cannabinoid compounds, most notably **Δ^9-tetrahydrocannabinol** (THC; Howlett *et al.*, 2002). This acts *via* the **cannabinoid CB_1 receptor** that is widely expressed by CNS nerves, to induce a number of behavioural and physiological effects (Howlett *et al.*, 2002). More recently, synthetic CB_1 receptor agonists, which often exhibit markedly higher agonist activity than THC (K_i = 40 nM, at CB_1 receptors; Howlett *et al.*, 2002), have become increasingly widely used as an alternative to botanical cannabis (Keyes *et al.*, 2016). These recreational drugs, such as Spice, Black Mamba and Buzz are laced with a variety of ever-changing, synthetic CB_1 receptor agonists (Fattore and Fratta, 2011; Hutter *et al.*, 2012; Hess *et al.*, 2015; Kemp *et al.*, 2016; Tournebize *et al.*, 2017). These agonists include JWH-018 (K_i = 9 nM), JWH-122 (K_i = 1 nM), JWH-250 (K_i = 11 nM), JWH-251 (K_i = 29 nM) and AM2201 (K_i = 1 nM), which can cause substantial intoxication, withdrawal symptoms, psychosis and death (Fattore and Fratta, 2011; Kemp *et al.*, 2016; Tournebize *et al.*, 2017). Although most exposures to these recreational drugs result in non-life-threatening effects, not requiring treatment (Hoyte *et al.*, 2012), those containing synthetic cannabinoids are causing an increasing number of apparently cannabinoid-related deaths (Hoyte *et al.*, 2012; Kemp *et al.*, 2016; Tournebize *et al.*, 2017).

In animals, cannabimimetic effects have been associated with a tetrad of behavioural effects including catalepsy, analgesia, lack of locomotor activity and thermoregulation, mediated mainly by THC within cannabis and by CB_1 receptors expressed within the CNS (Zimmer *et al.*, 1999; Varvel *et al.*, 2005; Croxford *et al.*, 2008). These behavioural effects induced by THC and synthetic cannabinoids can be blocked by CB_1 receptor antagonists (Varvel *et al.*, 2005; Marshell *et al.*, 2014). Likewise, behavioural and physiological effects of cannabis can be blocked by CB_1 receptor antagonists/inverse agonists in humans (Huestis *et al.*, 2007). Therefore, receptor blockade could act as an antidote to limit potentially life-threatening intoxication.

Although there are claims that inverse agonists of CB_1 receptors can reverse cannabimimetic effects of synthetic cannabinoids (Taffe *et al.*, 2015), on closer analysis, it is evident that these antagonists/inverse agonists are typically applied before the cannabinoid agonist. Therefore, it is an inhibition of the development of cannabimimetic effects rather than a reversal of established cannabimimetic effects (Huestis *et al.*, 2007; Marshell *et al.*, 2014; Taffe *et al.*, 2015). We therefore assessed the possibility that cannabimimetic effects of a synthetic cannabinoid could be reversed after they are manifest, to test the hypothesis that CB_1 receptor antagonists could act as antidotes to cannabinoid intoxication.

Methods

Animals

All animal care and experimental procedures were performed following ethical review by the Local Animal Welfare and Ethical Review Bodies and the UK Government Home Office. Animals were housed, and experiments were performed, in accordance with the Animals (Scientific Procedures) Act 1986 and European Union Directives EU 2010/63/EU. Animal studies are reported in compliance with the ARRIVE guidelines (Kilkenny *et al.*, 2010; McGrath and Lilley, 2015). Adult Biozzi Antibody High (ABH) female mice were from stock bred at Queen Mary University of London (Pryce *et al.*, 2014). As both male and female mice develop cannabimimetic effects (Pryce *et al.*, 2014), as found in humans (Schneir *et al.*, 2011; Tournebize *et al.*, 2017), animals were selected based on availability and knowledge of drug responsiveness in the ABH wild-type and ABH.*Cnr1*$^{-/-}$ CB_1 receptor-deficient mice (Pryce *et al.*, 2014).

Treatments

A dose of CB-13 (5 mg·kg^{-1} i.p.) was selected that was known to induce hypothermia in ABH mice and not in CB_1-deficient ABH mice (Pryce *et al.*, 2014). **AM251** was used at a dose (5 mg·kg^{-1}) known to prophylactically antagonize CB_1 agonists and was injected i.v. 20 min after CB-13 administration at a time when it was known that hypothermia and sedation would be present (Pryce *et al.*, 2014).

Temperature measurement

A K-type thermocouple was placed under the hind limb, and the maximum temperature at each time point was measured (Pryce *et al.*, 2014). This element of the tetrad tests (Varvel *et al.*, 2005) was selected as it could most easily, rapidly and repeatedly be measured in groups of animals. Sedation seen by visually assessed marked hypomotility was recorded as being evident or unremarkable, but was not measured using open-field monitoring of hypomotility within 5 min (Varvel *et al.*, 2005), as it was not possible to repeatedly quantitatively measure hypomotility with available equipment. Animals were randomly selected to treatment, and the study was unblinded. The sample size ($n \geq 5$) was based on experience from previous studies with other compounds to obtain adequate safety data to achieve the objectives of the study. Temperatures were measured at baseline, 20 min (Pryce *et al.*, 2014) and 60 min after administration of CB-13. Additional measurements at 40 and 120 min were taken in animals receiving AM251.

Data and statistical analysis

The data and statistical analysis comply with the recommendations on experimental design and analysis in pharmacology (Curtis *et al.*, 2015) Repeated measures one-way ANOVA, with Student–Newman–Keuls *post hoc* test, or ANOVA with Bonferroni *post hoc* test or *t*-tests, with the samples assessed for normality and equality of variances were assessed using Sigmaplot V11 (Systat Software Inc., Hounslow, UK). Paired analysis of the presence, assigned a value of 1, or absence assigned a value of 0, of visible marked sedation (hypomotility) was performed using repeated ANOVA on ranks using Sigmaplot V11. $P < 0.05$ was the level of statistical significance.

Materials

CB-13 (1-naphthalenyl[4-(pentyloxy)-1-naphthalenyl]methanone, a synthetic CB_1 agonist (K_i = 15 nM, EC_{50} = 6.1 nM at CB_1 receptors), and AM251, a CB_1 receptor

antagonist/inverse agonist (K_i = 8 nM), were purchased from Tocris (Bristol, UK) and dissolved in dimethyl sulphoxide : cremaphor: PBS (1:1:18).

Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Southan *et al.*, 2016), and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 (Alexander *et al.*, 2015a,b).

Results

As anticipated, a 5 mg·kg^{-1}, i.p. dose of CB-13 induced hypothermia in ABH mice (Figure 1), which has been shown previously to be CB_1 receptor-mediated and completely absent in CB_1 receptor-deficient mice (Pryce *et al.*, 2014). This induced significant visible sedation and also induced hypothermia, which was measured to provide a quantitative readout. The hypothermic effect was rapidly antagonized with AM251 (5 mg·kg^{-1}, i.v.; Figure 1), and the significant marked sedation, associated with the relative lack of motility, was lost within 20 min. The hypothermia was lost by 40 min after treatment with AM251 (Figure 1). Therefore, a CB_1 receptor inverse agonist can reverse CB_1 receptor-mediated cannabimimetic effects.

Discussion

This study suggests that CB_1 receptor inverse agonism/antagonism could act as an antidote to reverse cannabinoid

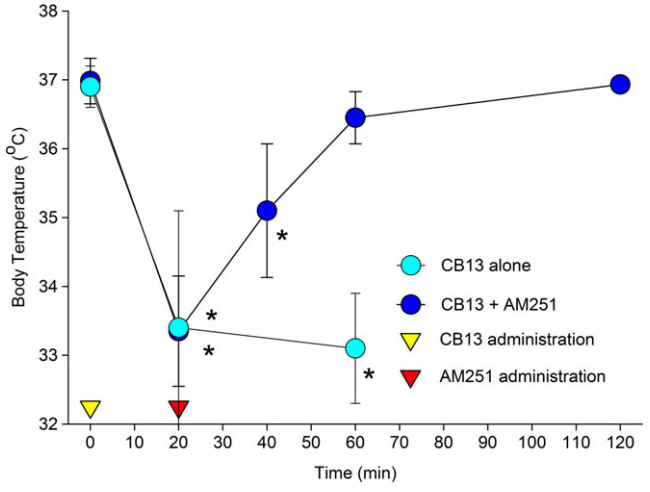

Figure 1

The CB_1 receptor antagonist AM251 reversed the hypothermic effects of the CB_1 receptor agonist CB-13. Animals (n = 6) were injected i.p. with CB-13 (5 mg·kg^{-1}) at 0 min, and AM251 (5 mg·kg^{-1}) was injected i.v. at 20 min. Temperature was assessed with a thermocouple placed under the hindlimb. Data shown are the group means ± SD. *P<0.05, significantly different from baseline values.

intoxication. However, the commercial development of CB_1 receptor antagonists, including studies with **rimonabant** (CB_1 K_i = 12 nM), **taranabant** (CB_1 K_i = 9–10 nM) and **otenabant** (CB_1 K_i = 1 nM) (Howlett *et al.*, 2002), was halted due to adverse neuropsychiatric effects (Janero and Makriyannis, 2009). However, many thousands of people have safely taken and tolerated a dose of a CB_1 receptor antagonist /inverse agonist (Van Gaal *et al.*, 2008; Topol *et al.*, 2010). The adverse events, notably depression, anxiety and a low risk of suicide, which prompted withdrawal of rimonabant from the market (Janero and Makriyannis, 2009), were not considered to be acceptable to justify its long-term use against what may be considered lifestyle, food and tobacco issues (Doggrell, 2008; Janero and Makriyannis, 2009). However, single-use cannabinoid antagonist therapy to block potentially life-threatening, cannabinoid intoxication may be worth the re-manufacture and testing for such an indication.

Although intoxication and possibly deaths (Hess *et al.*, 2015; Lusher, 2016) may be related to cannabinoid receptor agonism, as the causative agents are unlicensed, lack proper toxicology testing and have variable content, these deaths may relate to the actions of toxic metabolites on alternative targets unrelated to the cannabinoid system or possibly non-cannabinoid compounds. The physical effects usually reported include tachycardia, nausea, somnolence, hallucinations, paranoia and dry mouth syndrome (xerostomia) typical of cannabis intoxication. However, atypical cannabis intoxication effects and worse complications such as psychosis, seizures, flaccid paralysis, renal injuries, aggressiveness, cerebral ischaemia, cardiac arrhythmias, myocardial infarction, coma and death have all been reported following the use of synthetic cannabinoids (Kemp *et al.*, 2016; Tournebize *et al.*, 2017). Cannabis intoxication is not usually fatal in humans (Hartung *et al.*, 2014), but high doses of cannabinoids can cause death in animals, *via* cardiovascular effects that have been seen in people using synthetic cannabinoids (Beaulieu, 2005; Andonian *et al.*, 2017). Because there is not enough data to be confident that the toxicity of the 'Spice' products is really due to their cannabinoid content, perhaps the best way of determining whether this is the case would be to undertake a trial of a rescue cannabinoid receptor antagonist.

Proof of concept studies of p.o. formulations, for which there is toxicological data and knowledge from human use, could be administered to people who are conscious and compliant. This may help determine whether the investment required to develop an i.v. formulation for use by paramedics or more importantly an i.m. formulation for use in an emergency autoinjector is justified. Because of the knowledge associated with their use in humans, it would be easier to develop one of the antagonists/inverse agonists that have entered clinical development, notably rimonobant, as it would have a large history of human use and is known to antagonize some of the chemical entities found in 'Spice' (Hruba and McMahon, 2017). Further studies would be needed to determine whether other antagonists (McPartland *et al.*, 2015), which may lack the neurobehavioural issues associated with inverse agonism with compounds of high CB_1 receptor affinity, are of value. However, unless a CB_1-receptor antagonist manufacturer is willing to undertake such studies, it would

be futile to perform more animal studies. These could establish whether inverse agonism is required or whether receptor blockade by low-affinity antagonists, neutral antagonism or allosteric receptor modulation has similar efficacy. Just as naloxone can be used to limit the effects of opioid overdose (Wermeling, 2015), single-use CB_1 receptor inverse agonists could perhaps help save human life.

Acknowledgements

The authors thank the Multiple Sclerosis Society.

Author contributions

G.P. and D.B. contributed in the experimental concept and design, data analysis and manuscript drafting. G.P. also contributed in the *in vivo* experimentation.

Conflict of interest

The authors declare no conflicts of interest.

Declaration of transparency and scientific rigour

This Declaration acknowledges that this paper adheres to the principles for transparent reporting and scientific rigour of preclinical research recommended by funding agencies, publishers and other organisations engaged with supporting research.

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