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Antidote to cannabinoid intoxication

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Running Title: Cannabinoid antidote

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ABSTRACT

BACKGROUND & PURPOSE. Cannabis is a re-creational drug leading to intoxication, due to cannabinoid receptor one (CB_1) stimulation. However, more recently herbs mixed with synthetic cannabinoids sometimes known as "Spice" and "Black Mamba" have been increasing used and their high CB_1 receptor affinity means not only marked intoxication, but life-threatening complications and an increasing number of deaths. Whilst many studies have indicated that prophylactic CB_1 receptor antagonism can block cannabimimetic effects in animals and humans. The aim of the study was to determine whether CB_1 antagonism could reverse physical cannabimimetic effects.

EXPERIMENTAL APPROACH: Cannabimimetic effects, measured by hypothermic effects following sedation and hypomotility, were induced by 1-Naphthalenyl[4-(pentyloxy)-1-naphthalenyl] methanone (synthetic CB_1 agonist)in Biozzi ABH mice. *N*-(Piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (AM251. CB_1 antagonist) was subsequently administered and the influence on cannabimimetic effects assessed.

KEY RESULTS. In this study, the pre-existing, central nervous system-related cannabimimetic effects, measured via the hypothermic effect, induced by CB_1 receptor agonism where therapeutically treated and were rapidly reversed by CB_1 receptor antagonism/inverse agonism.

CONCLUSIONS & IMPLICATIONS. Cannabinoid receptor antagonists have been used in thousands of people and so may provide a single-dose antidote to cannabinoid intoxication, which may save human life.

ABBREVIATIONS

cannabinoid receptor one (CB_1)

KEY WORDS:

Cannabis, cannabinoid receptor, hyperthermia, spice, synthetic cannabinoids

INTRODUCTION

Cannabis sativa is a mind-altering re-creational drug that contains cannabinoid compounds, most notably Δ^9-tetrahydrocannabinol (Howlet et al., 2002). This acts via cannabinoid receptors that are widely expressed by the central nervous system, to induce a number of behavioural and physiological effects (Howlett et al., 2002). More recently synthetic CB_1 receptor agonists, which exhibit markedly higher agonist activity than THC (Howlett et al., 2002) have become increasingly prevalent as an alternative to botanical cannabis (Keyes et al., 2016). These drugs, such as Spice, Black Mamba and Buzz are laced with a variety of synthetic CB_1 cannabinoid receptor agonists (Fattore & Fratta 2011) that cause substantial intoxication, withdrawal symptoms, psychosis and death (Fattore & Fratta 2011). While, most (>90%) exposure result in non-life-threatening effects not requiring treatment (Hoyte et al., 2012), synthetic cannabinoids are causing an increasing number of cannabinoid-related morbidity (Hoyte et al., 2012).

In animals, cannabimimetic effects have been associated with a tetrad of behavioural effects including: catalepsy, analgesia, lack of locomotor activity and thermo-regulation, mediated mainly by Δ^9-tetrahydrocannabinol within cannabis and the CB_1 cannabinoid receptor, expressed within the central nervous system (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 (Varel et al., 2005, Marchell et al., 2014). Likewise, behavioural and physiological of cannabis can be blocked by CB_1 receptor antagonism/inverse agonism in humans (Huestis et al., 2007; Huestis et al., 2007). Therefore blockade could potentially act as an antidote to limit life-threatening intoxication.

Although there are claims that CB_1 receptor inverse agonism can reverse cannabimimetic effects of synthetic cannabinoids (Taffe et al., 2015), on closer analysis it is evident that antagonism/inverse agonism is typically applied before subsequent 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, Marchell et al., 2014, Taffe et al., 2015). We therefore addressed whether cannabimimetic effects of a synthetic cannabinoid can be reversed after they are manifest, to test the hypothesis, that CB_1 receptor antagonists can have antidote capabilities.

MATERIALLS & METHODS

Mice: Adult Biozzi ABH female mice were from stock bred at Queen Mary University of London (Pryce et al., 2014). Animal work was performed following ethical review by the Local Animal Welfare and Ethical Review Bodies and the UK Government Home Office. Animals were housed and experiments performed, in accordance with the Animals (Scientific Procedures) Act 1986 and European Union Directives EU 2010/63/EU.

Chemicals: 1-Naphthalenyl[4-(pentyloxy)-1-naphthalenyl]methanone (CB13) a synthetic CB1 cannabinoid receptor (EC_{50} values are 6.1nM) agonist and *N*-(Piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (AM251) a CB_1 receptor (EC_{50} values are 8nM) antagonist/inverse agonist were purchased from Tocris (Bristol, UK) and dissolved in dimethyl sulphoxide:cremaphor: phosphate buffered saline (1:1:18).

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 and rapidly measured in groups of animals. Animals were randomly selected to treatment, the study was unblinded. The sample size was based on experience from previous studies with other compounds to obtain adequate safety data to achieve the objectives of the study.

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 analysis of variance, with Student-Newman-Keuls post-hoc test, or analysis of variance 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). $P<0.05$ was the level of statistical significance

RESULTS

A 5mg/kg intra-peritoneally dose of 1-Naphthalenyl[4-(pentyloxy)-1-naphthalenyl] methanone was selected that was known to induce hypothermia in ABH mice (Table 1). This occurs via a CB_1 receptor-mediated effect, as the hypothermic response was completely lost in CB_1 receptor-deficient mice (Pryce et al. 2014). This induced visible sedation and hypothermia (Table1. Figure 1). This effect was rapidly antagonised with 5mg/kg i.v. (Table1. Figure 1) and visible sedation was lost within 20minutes and the hypothermia was lost by 40mins after CB1 antagonism (Table1. Figure 1). Therefore, a CB_1 receptor inverse agonist can reverse CB_1-mediated cannabimimetic effects.

DISCUSSION

This study suggests that CB_1 receptor inverse agonism/antagonism could act as an antidote to reverse cannabinoid intoxication. However, the commercial development of CB_1 receptor antagonism, including studies with: rimonabant, taranabant and otenabant, was halted due to neuro-psychiatric, adverse effects (Janero & Makriyannis 2009). Yet, many thousands of people have safely taken and tolerated a dose of CB_1 receptor antagonism/inverse agonism (Van Gaal et al. 2008, Topol et al. 2010). Whilst the adverse events: notably depression anxiety and low risk of suicide that prompted withdrawal of rimonabant from the market (Janero & Makriyannis 2009), where not considered sufficiently safe for long-term use against, what may be considered lifestyle, food and tobacco, issues (Doggrell 2009; Janero & 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. Whilst intoxication and deaths (Lusher 2016) are probably related to cannabinoid receptor agonism, as the agents are unlicensed and lack proper toxicology testing, the deaths may relate to actions on alternative targets. However, unless a CB_1-receptor antagonist manufacturer is willing to undertake such studies, it would be futile to perform more animal studies. Just as naloxone can be used to limit the effects of opioid overdose (Wermeling 2015), single use CB_1 receptor inverse-agonism could perhaps help save human life.

AUTHOR CONTRIBUTIONS: Experimental Concept & Design: GP, DB; *In Vivo* Experimentation: GP; Data Analysis: GP, DB; Manuscript Drafting DB, GP

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COMPETING INTERESTS STATEMENT: None relevant.

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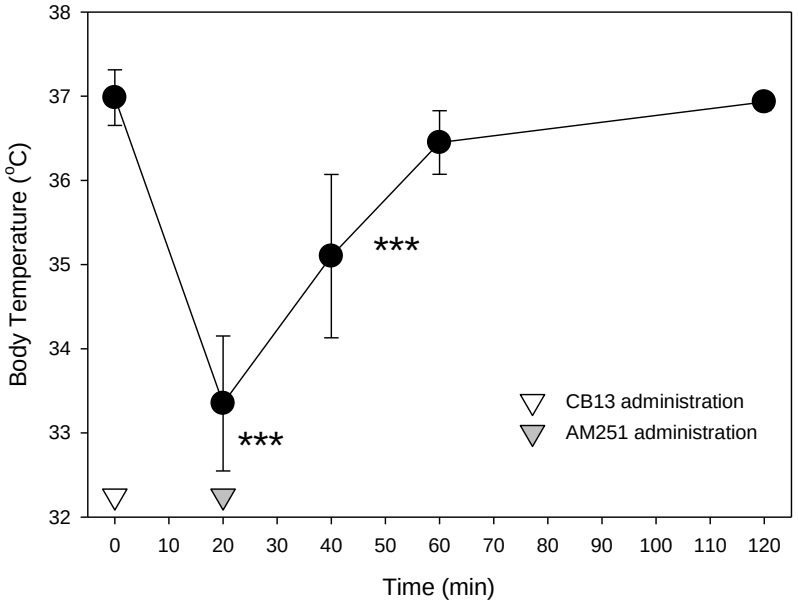
FIGURE 1. CB_1 receptor antagonism can reverse hypothermic effects.



Animals (n=6) were treated with 5mg/kg i.p. CB13 at 0min and 5mg/kg i.v. AM251 was injected at 20min. Temperature was assessed using a thermocouple placed under the hindlimb. Data represents the group mean ± standard deviation. *** P<0.001 compared to baseline.

TABLE 1. CB_1 receptor antagonism can reverse hypothermic effects.

	CB13			CB13 + AM251			
Time	n	Temp (°C)	P vs. Time	n	Temp (°C)	P vs. Time	P vs. CB13
0min	7	36.9 ± 0.3		6	37.0 ± 0.3		n.s.
20min	7	33.4 ± 1.7	P<0.001	6	33.4 ± 0.8	p<0.001	n.s.
60min	7	33.1 ± 0.8	P<0.001	6	36.5 ± 0.4	n.s.	P<0.001

Animals were treated with 5mg/kg i.p. CB13 at 0min and 5mg/kg i.v. AM251 was injected at 20min. Temperature was assessed using a thermocouple placed under the hindlimb. Data represents the group mean ± standard deviation. Not significantly different n.s.