Naltrexone does not attenuate the effects of intravenous Δ⁹-tetrahydrocannabinol in healthy humans

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Abstract

Although a wealth of preclinical evidence indicates an interplay between the μ-opioid (MOR) and cannabinoid 1 receptor (CB1R) systems, the precise nature of the cross modulation in humans is unclear. The objective of this study was to evaluate the effects of pretreatment with the MOR antagonist, naltrexone, on the subjective, behavioural and cognitive effects of the CB1R agonist, Δ⁹-tetrahydrocannabinol (THC), in healthy human subjects. Healthy human subjects, screened carefully for any medical or psychiatric illness, were administered either placebo or active naltrexone (25 mg) orally on each test day, followed 45 min later by placebo and 165 min later by active i.v. THC (0.025 mg/kg) in a randomized, fixed-order, double-blind manner. Subjective, behavioural and cognitive effects were assessed before and at several points after each drug administration. THC produced expected effects, including euphoria, anxiety, transient perceptual alterations, transient psychotomimetic effects and cognitive impairments. However, naltrexone did not produce any effects alone, nor did it attenuate any of THC's effects. Thus, in healthy human subjects who use cannabis intermittently, MOR antagonism does not modulate the common acute subjective, behavioural and cognitive effects of THC.

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Introduction

Abundant preclinical evidence suggests a close interaction between the endogenous cannabinoid (CB) and μ-opioid systems. Cannabinoid receptors (CB₁Rs) and μ-opioid receptors (MORs) belong to the same family of G-protein coupled receptors (Howlett et al. 2002; Pickel et al. 2004; Rios et al. 2006), are co-localized in a number of brain regions, including the nucleus accumbens core and shell, the locus coeruleus and the dorsal horn of the spinal cord (Pickel et al. 2004; Rodriguez et al. 2001; Salio et al. 2001; Vigano et al. 2005) and agonists at both receptors have reinforcing properties and are drugs of abuse.

Functional cross modulation between the two systems has long been recognized and preclinical studies demonstrate a synergistic effect of CB₁R and MOR agonists on a wide range of behavioural measures (Manzanares et al. 1999b; Massi et al. 2001), endocrine responses (Manzanares et al. 1999a) and neurochemical (Tanda et al. 1997) and cellular (Rios et al. 2006) responses. The cross modulation between the CB and MOR systems is particularly prominent with regard to their anti-nociceptive and rewarding effects. Thus, agonists at CB₁Rs induce a release of endogenous opiates to produce analgesia (Welch & Eads, 1999); a combination of CB₁R and MOR agonists produces synergistic analgesic effects in rats (Welch & Stevens, 1992; Welch et al. 1995); CB₁R antagonists reverse the analgesic effects of MOR agonists, while increasing...
endogenous CB levels enhance the analgesic effects of MOR agonists (da Fonseca Pacheco et al. 2009).

Many agonists at CB₁R and MOR are drugs of abuse with a common putative reward mechanism that is thought to be mediated by dopamine release in the nucleus accumbens (Chen et al. 1990; Tanda et al. 1997). Δ⁹-tetrahydrocannabinol (THC), a partial agonist at CB₁R, has been demonstrated to cause a release of endogenous opiate peptides (Pugh et al. 1996; Valverde et al. 2001) and to interact with opioid receptors in a non-competitive manner (Vaysse et al. 1987). Pretreatment with THC induced sensitization to the acute rewarding effects of opiate agonists, especially in rats vulnerable to addictive behaviours (Madon et al. 2001; Lamarque et al. 2001). Similarly, pre-exposure to morphine (an opiate agonist) induced sensitization to the acute effects of THC (Madon et al. 2001). Further, increasing levels of the endogenous CB, anandamide, attenuated opiate withdrawal (del Arco et al. 2002), while CB antagonist treatment reduced opiate self-administration and induced withdrawal in morphine-dependent rats (Navarro et al. 1998, 2004).

Consistent with the above, MOR agonists increased the reinforcing effects of CB₁R agonists in animals (Fattore et al. 2004; Justinova et al. 2008; Spano et al. 2004), while MOR antagonists naloxone and naltrexone reduced them (Braida et al. 2004, 2001; Justinova et al. 2004; Navarro et al. 2001). Naloxone also precipitated a mild CB withdrawal-like syndrome in CB-dependent rats (Navarro et al. 2001), suggesting opioid involvement in the development of CB₁R dependence. Furthermore, in animals perinatally exposed to THC, naloxone induced withdrawal responses similar to those observed in opiate-dependent rats (Corchero et al. 1998b), suggesting that THC exposure may impact the developing opiate system, possibly involving changes in proenkephalin gene expression (Corchero et al. 1997, 1998a, 1999a, 2002; Perez-Rosado et al. 2000).

In summary, substantial preclinical data support the notion that the endogenous MOR system may modulate and be involved in the rewarding effects of CB₁R agonists and CB dependence (tolerance/withdrawal). While there is significant preclinical data suggesting overlap between the CB₁R and MOR systems, there are some inconsistencies in the literature suggesting that this overlap is incomplete. For example, MOR agonists are discriminated from THC in rodents, non-human primates and in humans (Li et al. 2008; Lile et al. 2009; Solinas & Goldberg, 2005; Solinas et al. 2004). Other data in rodents as well as non-human primates suggest that MOR antagonists fail to block the reinforcing effects of CB₁R agonists and do not induce withdrawal in CB-treated animals (Beardsley et al. 1986; Wakley & Craft, 2011).

A limited number of studies have examined the effects of opioid antagonism on the rewarding effects of THC in humans and found mixed results. Naltrexone (50 mg) pretreatment had no effect on oral THC-induced dose dependent (7.5 and 15 mg) subjective and behavioural effects in 14 healthy intermittent cannabis users (Wachtel & de Wit, 2000). Haney et al. published a series of studies on the interactive effects of naltrexone pretreatment and oral and smoked THC in heavy daily cannabis users and non-users (Cooper & Haney, 2010; Haney, 2007; Haney et al. 2003). One early study found that naltrexone (50 mg) potentiated the euphoria induced by oral THC (30 mg) in heavy users (Haney et al. 2003), but a later study found that a lower dose of naltrexone (12 mg) blunted the rewarding effects of low dose oral THC (20 mg) in heavy users while enhancing the effects of a very low dose (2.5 mg) in a small sample of non-users (Haney, 2007). A subsequent study of a wide range of naltrexone doses (0–100 mg) found that naltrexone enhanced the rewarding effects of smoked THC in heavy daily users of cannabis (Cooper & Haney, 2010). It is noteworthy that in most of these studies, THC alone did not produce any effect on a number of cognitive measures, such as divided attention, digit or word recall or the digit symbol substitution task (DSST), suggesting that either the dose of THC was too low or that subjects were tolerant to the effects of THC. Naltrexone, however, did impair vigilance, performance on the DSST and delayed recognition recall by itself and also in combination with THC. Most of these studies were conducted in very heavy daily cannabis users, who likely met criteria for cannabis dependence. In this regard, as discussed below, it is important to note that preclinical data demonstrate alterations in the opioid system secondary to chronic CB₁R agonist exposure (Madon et al. 2001; Corchero et al. 1999a).

Thus, the human data on the naltrexone × THC interactions are also mixed and their interpretation is complicated by a number of factors. First, most of the human data are from heavy and daily cannabis users, who may have alterations in their MOR systems. Second, heavy cannabis users are also likely to be tolerant to the effects of THC. Third, given the wide range of doses used and the oral or smoked routes of THC administration, it is unclear how the pharmacokinetic interactions between THC and naltrexone may influence the behavioural results. Fourth, in some
studies, subjects were permitted to continue smoking tobacco cigarettes during the test day; while this avoided nicotine withdrawal, the nicot ine×MOR (Hadjiconstantinou & Neff, 2011) or nicotine×THC interactions (le Foll et al. 2006) were unaccounted for. Finally, it is important to note that opioid mechanisms are not involved in all the effects of CBs; for instance, the catalepsy produced by THC was unaffected by naloxone but eliminated by anticholinergic pretreatment (Frescott et al. 1992). Similarly, the mydriatic effect of THC was not influenced by naloxone (Korczyn & Eshel, 1982). These results demonstrate that distinctive mechanisms of action exist for some CB-induced behaviours that are not affected by opiate antagonists.

CB₁R agonists produce a wide range of subjective, cognitive and behavioural effects. Human studies conducted on naltrexone×THC interactions thus far have focused primarily on subjective effects. Further, the route of THC administration in these studies, i.e. oral and inhaled, has variable intra- and inter-individual pharmacokinetics (Azorlosa et al. 1995, 1992; Grotenhermen, 2003). The majority of studies used oral THC, which has poor bioavailability and significant pharmacokinetic variability (Ohlsson et al. 1980). Therefore, oral THC may not be the best way to study the interactions of the MOR and CB₁R systems in humans. The smoked route of THC administration, while more reliable than the oral route, is also associated with substantial intra- and inter-individual variability (d’Souza et al. 2004; Lindgren et al. 1981). Furthermore, the effects of other components of smoke that is inhaled when smoking a THC-containing cigarette are not accounted for in studies in which THC was administered by smoking.

The goal of this study was to examine the effects of a single dose of oral naltrexone on a wide range of subjective, behavioural, psychotomimetic and cognitive effects of i.v. THC in individuals with limited current and past exposure to cannabis, using a well-validated paradigm that reliably produces transient physiological, subjective, behavioural and cognitive effects. The route of THC administration was i.v., which minimizes the inter- and intra-individual variability in absorption and standardizes the THC dose in subjects. The subjects in this study were light and intermittent cannabis users who did not meet criteria for current or past cannabis dependence and may be most representative of the pattern of cannabis use in the general population. Finally, a wider range of outcomes assessing the effects of opiate antagonism on the psychotomimetic and cognitive effects of THC, which have not been adequately studied (Greenwald & Stitzer, 2000; Wachtel & de Wit, 2000), were included in this study.

We hypothesized that pretreatment with naltrexone would blunt the acute euphoric and rewarding effects of i.v. THC in healthy subjects without having any significant ameliorative effects on the psychotomimetic symptoms or cognitive deficits induced by THC.

Method

This study was approved by the Institutional Review Boards at Yale University (Human Investigations Committee, USA) and the VA Connecticut Healthcare System (Human Subjects Subcommittee, USA). The study was conducted at the Neurobiological Studies Unit (VA Connecticut Healthcare System, USA).

Screening

After obtaining written informed consent, subjects (18–55 yr) underwent a structured psychiatric interview for DSM-IIIIR or IV (First et al. 2002) and were carefully screened for any DSM Axis I or Axis II lifetime psychiatric or substance use disorder and family history of major Axis I disorder. All subjects were asked to estimate their lifetime cannabis exposure (no. of times), heaviest exposure and last exposure to cannabis. Subjects were excluded for recent abuse (3 months) or dependence (1 yr) on alcohol or any substances other than nicotine. Only tobacco smokers who were able to abstain for about 12 h (the typical length of a test day) were allowed to participate. Cannabis-naïve individuals were excluded to minimize any risk of promoting future cannabis use/abuse. The history provided by subjects was confirmed by a telephone interview conducted with an individual (spouse or family member) identified by the subject prior to screening. A general physical and neurological examination, electrocardiography and laboratory tests (serum electrolytes, liver function tests, complete blood count with differential and urine toxicology) were also conducted. Subjects were instructed to refrain from alcohol, illicit drugs or prescription drugs not approved by the research team for 2 wk before the study and throughout study participation.

Experimental design (Table 1)

Subjects reported to the test facility at approximately 08:30 hours on the morning of each of the two test days. Test days lasted until around 15:00 hours. On each test day they received placebo or active (25 mg) p.o. naltrexone in double-blind, randomized,
counterbalanced order, followed 45 min later by placebo THC (vehicle) and 165 min later by 0.025 mg/kg active THC administered i.v. over 20 min (approximately equal to 1.75 mg in a 70 kg individual).

Although the study had a fixed order of THC administration, subjects and raters were kept blind to the fixed order by an elaborate deception described in the supplementary section (available online).

**Drugs**

The dose and rate of administration of THC was chosen to mimic the dose range of recreational cannabis use.
and to be equivalent to about 0.5–1.5 standard National Institute of Drug Abuse THC cigarette. A 20-min infusion was selected to mimic the time frame of recreational cannabis consumption. The rationale for the route of THC administration and preparation of both THC and placebo has been described previously (d'Souza et al. 2004). THC of 99.6% purity was dissolved in 95% ethanol (Agurell et al. 1986) to yield a concentration of 2 mg/ml stock solution, which was then passed through a 0.22 μm polymer filter, subjected to sterility and pyrogenicity testing, assayed by gas chromatography–mass spectrometry to confirm its concentration, and stored at −20 °C for future use. For the control condition, an equivalent volume (2 ml) of ethanol (vehicle) was used, which would amount to a concentration of 0.0004% in an adult with average blood volume (4–5 l). Post-infusion blood sampling at multiple time points failed to detect ethanol.

Naltrexone is a long-acting, non-selective MOR antagonist. Its major metabolite, 6-β-naltrexol, also an opioid antagonist, is believed to be responsible for the long duration of effects of naltrexone. Naltrexone has highest affinity for MOR and is a competitive antagonist at μ, δ and κ-opioid receptors. Naltrexone attenuates opiate effects such as euphoria (O’Brien et al. 1984; Webster et al. 2011) and analgesia (Bhargava et al. 1993) and is approved for the treatment of opiate dependence (Guthrie, 1990). Previous studies employing this dose of oral naltrexone have been well tolerated by healthy subjects (Krystal et al. 2006). Naltrexone is rapidly absorbed orally and 50 mg p.o. achieves peak plasma concentrations (43.6 ng/ml) within 1 h of oral administration. At 2, 4 and 8 h, plasma concentrations are 36.2, 20.2 and 8.0 ng/dl. Plasma naltrexone concentrations of 2 ng/dl have been associated with >85% blockade of 25 mg i.v. heroin, demonstrating substantial opioid receptor antagonism at blood levels of 2 ng/ml. These data suggest that following administration of a single dose of 25 mg naltrexone, significant opioid receptor blockade will begin within 30–60 min and persist for several hours. Thus, the active THC infusion at +165 min was timed to occur well within the duration of effects of the oral naltrexone administration.

**Outcome measures**

**Subjective effects**

*Marijuana Effects Scale.* Effects of marijuana were assessed using a 100 mm visual analogue scale (VAS). Feeling states associated with marijuana intoxication (‘high’, ‘calm and relaxed’, ‘hungry’, ‘tired’, ‘anxious’, etc.) were selected from the marijuana group pattern of the Addiction Research Center Inventory and the Belleville’s Marijuana Scale (Haertzen, 1965, 1966). Subjects were instructed to rate each item according to his/her perceived intensity of experience at several time points during each test day.

*Marijuana craving.* A modified craving scale adapted from Tiffany & Drobes (1991) was administered at various time points during each test day. Items on the scale included: (1) desire to consume; (2) intention to consume; (3) sense that consumption would result in feeling better; (4) sense that consumption would reduce discomfort; (5) sense of control over consumption.

*Drug-liking.* Drug-liking was measured at various time points on each test day using a Likert scale (0 to 100 mm; 0 = not at all, 100 = extremely).

*Drug choice.* On completion of both test days, subjects were asked to rate the conditions in order of their choice/preference.

**Similarity to cannabis scale.** Twice on each test day (at the end of the placebo THC condition and the active THC condition), subjects were asked to rate the similarity of the drug experience to their recreational cannabis use using a Likert scale (0–100 mm; 0 = not at all, 100 = extremely).

**Behavioural effects**

**Perceptual alterations**

Alterations in perception of the environment, time and body, feelings of unreality or dissociation, memory impairment, etc. were measured using the Clinician Administered Dissociative Symptoms Scale (CADSS; Bremner et al. 1998; Krystal et al. 1994). The CADSS has subject (self)-rated and clinician-rated subscales and has been shown to be sensitive to THC effects (d’Souza et al. 2004, 2005, 2008a, b).

**Positive and negative psychotic symptoms**

Transient THC-induced positive and negative psychotomimetic symptoms were measured using the Positive and Negative Syndrome Scale (PANSS; Kay et al. 1989). A modified version of the PANSS, adapted for repeated measurements within a short time period, was used. This has been shown to be sensitive to THC-induced effects in a number of previous studies (d’Souza et al. 2001, 2005, 2008a, b).
Cognitive effects

The neuropsychological test battery was administered approximately 30 min after the end of placebo and active THC administration, i.e. when peak effects were expected. As noted below, a number of tasks were administered using the Cambridge Neuropsychological Test Automated Battery (CANTAB).

Verbal memory and recall

Vocal memory and recall was measured on the Hopkins Verbal Learning Test (HVLT), a word list learning measure of verbal memory and hippocampal function (Brandt, 1991; Brandt & Benedict, 1991), which has been shown to be sensitive to the acute effects of i.v. THC (d’Souza et al. 2005, 2008).

Response inhibition

Response inhibition was measured using the colour word task of the Stroop interference task, which has been shown to be sensitive to marijuana exposure (Hooker & Jones, 1987).

Sustained attention

Sustained attention was measured using the Rapid Visual Information Processing, from the CANTAB, which has been shown to be sensitive to THC effects. A’ was determined as the signal detection measure of sensitivity to errors, regardless of error tendency (range 0.00–1.00; bad to good).

Visual memory

Visual memory was measured on the delayed matching to sample task from the CANTAB, which assesses both simultaneous and short-term visual memory. The participant was shown a complex visual pattern (the sample) and then, after a brief delay, four similar patterns. The task was to correctly identify the pattern that matched the sample.

Working memory

Working memory was measured using the spatial working memory task, from the CANTAB, to test the subject’s ability to retain spatial information and to manipulate items in working memory. A number of coloured boxes were shown on the screen. The participant had to find blue tokens, one in each of the boxes, and use them to fill up an empty column on the right-hand side of the screen. Touching boxes already found to be empty during the same trial or revisiting boxes already found to contain a token were both errors. The number of boxes (load) was gradually increased to a maximum of eight.

Visuospatial recognition memory

Visuospatial recognition memory was tested using the spatial recognition memory task from the CANTAB. Participants were shown series of white squares in five locations during the presentation phase. During the test phase, five pairs of squares were shown in different locations, one of which matched a location during the presentation phase. Accuracy and latency to identify the matching location were the primary outcomes.

Orientation

Orientation was assessed using the Mini-Mental State Exam (Folstein et al. 1975) at various time points and at the end of each test day prior to clearing the subject for discharge.

Long-term follow-up assessment of cannabis use

All subjects who completed at least one test day in the study had safety follow-up contact at 1, 3, 6 and 12 months after their participation to explore any long-term consequences of THC exposure in the lab.

Data analysis

Initially, data were examined descriptively using means, S.D. and graphs. Each outcome was tested for normality using Kolmogorov–Smirnov test statistics and normal probability plots. All behavioural outcomes were highly skewed even after log transformation. Thus, these outcomes were analysed using the non-parametric approach for repeated measures data (Brunner et al. 2002), in which data are first ranked and then fitted using a mixed-effects model with an unstructured variance–covariance matrix and p values adjusted for analysis of variance-type statistics (ATS). Linear mixed models were used to analyse all cognitive outcomes, which were sufficiently normally distributed, except for delayed recall on the HVLT. In these models, each outcome represented the dependent variable, while naltrexone (placebo vs. active) and time were included as within-subject explanatory factors. Since placebo and THC were administered sequentially in fixed order, effects of ‘time’ reflect the effects of placebo or THC and are reported thus in the results. All models of CANTAB data were controlled for National Adult Reading Test IQ and motor function (latency). For verbal immediate recall, trial (1–3) was included as an additional within-subject factor. These models allowed for testing of all main and
interaction effects of naltrexone and THC. When appropriate, post-hoc comparisons were performed. Data were analysed using SAS, version 9.1 (SAS Institute, USA). Bonferroni correction was applied within but not across hypotheses; thus, for the positive symptoms subscale of the PANSS, a cut-off $a$ level of $0.05/3 = 0.016$ was used to declare effects significant for PANSS positive.

Results

Subject characteristics (Table 2)

Thirty subjects participated in the study, of which 26 completed all test days. Seven subjects had used cannabis in the past 2 wk while 23 had a remote history of exposure to cannabis.

Subjective effects (Table 3: supplementary section)

Marijuana subjective effects (Fig. 1)

As expected, THC administration significantly increased self-reported ratings on ‘high’ ($ATS = 45.4$, $d.f. = 4.03$, $p < 0.0001$; Fig. 1a), ‘tired’ ($ATS = 7.17$, $d.f. = 3.34$, $p = 0.033$), ‘calm’ ($ATS = 2.62$, $d.f. = 3.36$, $p = 0.004$) and ‘anxious’ ($ATS = 3.44$, $d.f. = 3$, $p = 0.016$; Fig. 1b) as measured by VAS. Naltrexone did not produce a significant effect on any of these items by itself, nor did it alter the response to THC on any of these subjective effects.

Marijuana craving

Administration of THC did not produce any significant changes on the marijuana craving questionnaire. Naltrexone did have a significant main effect on ‘liking’ ($ATS = 5.2$, $d.f. = 1$, $p = 0.023$) and ‘intending to use marijuana’ ($ATS = 5.38$, $d.f. = 1$, $p = 0.02$). There were no significant naltrexone $\times$ time or naltrexone $\times$ THC interactions. There were no significant interactive effects between naltrexone and THC.

‘Drug-liking’ and ‘drug choice’ (Fig. 2)

THC had a significant effect on all scores on the ‘drug liking’ questionnaire consistent with its rewarding effects. THC increased scores on ‘similarity of experience’ [to smoking marijuana ($ATS = 114.0$, $d.f. = 1$, $p < 0.0001$); ‘enjoyment’ ($ATS = 21.9$, $d.f. = 1$, $p < 0.0001$)] and how much subjects were ‘willing to pay’ for the experience ($ATS = 12.7$, $d.f. = 1$, $p = 0.0004$). Naltrexone alone did not produce any significant effects on these items, nor did it have any interactive effects with THC.

Behavioural effects (Table 3: supplementary section)

Perceptual alterations (Fig. 3)

THC produced robust increases in perceptual alterations measured by the CADSS. THC increased scores on the subject-rated ($ATS = 45.4$, $d.f. = 3.9$, $p < 0.0001$) as well as clinician-rated ($ATS = 47.2$, $d.f. = 3.06$, $p < 0.0001$) CADSS subscales. As expected, naltrexone had no significant main effects on the CADSS. There were no significant THC $\times$ naltrexone interactions on perceptual alterations.

Psychotomimetic symptoms (Fig. 4)

THC produced increases in psychotomimetic symptoms as measured by the PANSS total scores ($ATS = 45.5$, $d.f. = 3.72$, $p < 0.0001$). THC increased scores on PANSS positive ($ATS = 35.3$, $d.f. = 2.77$, $p < 0.0001$), PANSS negative ($ATS = 23.3$, $d.f. = 3.28$, $p < 0.0001$) and PANSS global ($ATS = 29.8$, $d.f. = 3.71$, $p < 0.0001$) scales. The magnitude of the THC-induced mean increases in PANSS total scores (5.7) and PANSS positive scores (2.25) is similar to that observed in other studies with lower doses of THC (d’Souza et al. 2008a, b). As expected, naltrexone had no significant main effects on any of these measures. Further, there were no significant THC $\times$ naltrexone interactions on psychotomimetic effects.
Attention and vigilance

THC did not produce significant effects on latency, ‘hits’ or sensitivity (A’). Naltrexone did have significant effects on both total ‘hits’ ($F_{1,66} = 5.66, \ p = 0.0202$) and sensitivity index A’ ($F_{1,66} = 5.45, \ p = 0.0225$) without affecting latency. These main effects were primarily due to a lower baseline (pre-THC) in the placebo naltrexone condition. There were no naltrexone $\times$ time or naltrexone $\times$ THC interactions.

Verbal memory

THC produced robust impairments in immediate ($F_{1,268} = 18.7, \ p < 0.0001$) and delayed (ATS = 3.97, d.f. = 1, $p = 0.046$) recall measured by the HVLT. Naltrexone alone did not affect verbal memory on this task, nor were there any interactive effects with THC.

Neither THC nor naltrexone produced any significant effects on the spatial working memory, delayed match to sample task and spatial recognition memory tasks on the CANTAB.
Neither THC nor naltrexone produced any effects on response inhibition measured on the ‘Stroop interference’ task.

Discussion

The main finding of this study was that oral naltrexone did not significantly affect the acute rewarding effects of THC. Furthermore, as hypothesized, naltrexone did not affect the perceptual altering, psychotomimetic or cognitive effects of i.v. THC in this sample.

The dose of THC in this study produced an expected range of subjective, behavioural and cognitive effects in this sample. Since the effects of THC were consistent with the known effects of recreational cannabis use and rated as similar to cannabis use by subjects, the findings of the study are relevant to recreational cannabis use. The dose of naltrexone was based on our experience in previous studies showing that 25 mg naltrexone pretreatment potentiated the

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**Cannabinoid–opioid interactions**

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**Response inhibition**

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psychotomimetic effects of a subthreshold dose of i.v. ketamine in healthy subjects (Krystal et al. 2006). The fact that 25 mg naltrexone had no effect on a wide range of THC-induced effects therefore suggests that, at least at this dose, the MOR system does not influence the effects of CB₂R agonism.

Our results are consistent with some reports (Greenwald & Stitzer, 2000; Wachtel & de Wit, 2000) but not others (Cooper & Haney, 2010; Haney, 2007; Haney et al. 2003). There are a number of differences between our study and those of Haney et al., which might contribute to the differences in the results. First, our sample included subjects who did have exposure to cannabis but much lower than most of the subjects studied by Haney et al., who were largely heavy and daily cannabis users. Heavy cannabis use may be associated with an altered response to THC secondary to the development of tolerance (d’Souza et al. 2008). Furthermore, heavy cannabis use may also be associated with alterations in the opioid system. Preclinical studies suggest that repeated daily administration of THC in rats produced behavioural sensitization to morphine, even after a gap of 14 d (Cadoni et al. 2001). This study demonstrated that prior repeated exposure to THC produced persistent alterations in the response to opiates, which was similar to the sensitization produced by prior exposure to morphine. Finally, daily administration of THC for 1–14 d in rats resulted in a persistent up-regulation of the proenkephalin gene (Corchero et al. 1999a, b).

Second, our study differed in the route of administration of THC. We administered i.v. THC while Haney et al administered oral and smoked THC. The route and rate of administration may influence the overall effects of THC and thereby the interactions between naltrexone and THC. Third, as reported in Table 2, most of our subjects were non-smokers and smoking was not permitted during our test days. Thus, our study minimized the potential confounding effects of nicotine and smoking on the main outcomes.

In summary, the clinical and preclinical literature on the interactions between the MOR and CB₂R systems suggests that a simple model of synergism between the two systems does not explain their complex interactions. Thus, although CB₂R agonists induce endogenous opiate release, this may not be their sole mechanism of action, since not all the effects of CB₂R agonists are attenuated by MOR antagonists. This may, in part, be related to the effects of CB₂R agonists on κ and δ opioid receptors and their endogenous ligands. Second, there are some data suggesting that CB₂R and MOR receptors form functional heterodimers (Hojo et al. 2008; Rios et al. 2006) and the consequences of acute/chronic CB exposure on such systems (such as receptor activation/down-regulation) need to be further explored. Finally, the interactions between the CB and opioid systems may not be direct but secondary to the modulation of dopamine release. The behavioural cross sensitization between the two systems has been shown to be associated with reduced dopamine transmission in the shell and heightened transmission in the core of the nucleus accumbens (Cadoni et al. 2008). Naloxone, a MOR antagonist, blocks both the THC- and heroin-induced dopamine efflux in the nucleus accumbens (Chen et al. 1990). These data suggest that CB₂R and MOR agonists may exert similar effects on mesolimbic dopamine transmission likely involving a common MOR-mediated mechanism located in the ventral mesencephalic tegmentum.

This study, however, did not examine the mechanisms involved in interactions between the CB and opioid systems.

**Strengths**

This double blind, randomized study involved intermittent users of cannabis; a sample that may be more representative of the typical pattern of recreational cannabis use. Further, studying this sample of subjects avoids the effects of chronic CB exposure on the CB₂R and MOR systems. As noted by Haney et al., the degree of previous cannabis exposure may influence naltrexone × THC interactions. The use of i.v. THC avoids intra- and inter-individual variability in THC doses, while the low dose and slow infusion over 20 min mimics the pharmacokinetics observed during smoking cannabis. Other strengths include the prohibition of smoking during test days and the inclusion of a wide range of subjective, behavioural and cognitive measures sensitive to THC.

**Limitations and future directions**

The limitations of this study include the use of a single dose of oral naltrexone and the fixed order or administration of the single dose of THC.

Future studies using a wide range of doses of i.v. THC and an i.v. opiate antagonist (such as naloxone) in subjects with a wide range of cannabis use may help eliminate some confounds present in the literature and clarify the interactions between the two systems. Further studies should also be directed at examining the effects of selective μ, κ and δ opiate antagonists on THC-induced effects to examine the interactions between the CB system and these opiate systems.
Note
Supplementary material accompanies this paper on the Journal’s website (http://journals.cambridge.org/pnp).

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