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Substitution profile of Δ⁹-tetrahydrocannabinol, triazolam, hydromorphone and methylphenidate in humans discriminating Δ⁹-tetrahydrocannabinol

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Abstract

Rationale—Preclinical evidence suggests that non-cannabinoid neurotransmitter systems are involved in the behavioral and physiological effects of cannabinoids, but relatively little research has been conducted in humans.

Objectives—The aims of this study were to assess whether oral Δ^9-tetrahydrocannabinol (Δ^9-THC) would function as a discriminative stimulus in humans and to examine the substitution profile of drugs acting at opioid, GABA and dopamine systems.

Methods—Healthy subjects who reported moderate cannabis use were enrolled. Subjects learned to identify when they received oral 25 mg Δ^9-THC or placebo under double-blind conditions. Once subjects acquired the discrimination (i.e., ≥ 80% drug-appropriate responding for four consecutive sessions), multiple doses of Δ^9-THC, the $GABA_A$ positive modulator triazolam, the μ-opioid agonist hydromorphone and the dopamine reuptake inhibitor methylphenidate were tested to determine if they shared discriminative-stimulus effects with the training dose of Δ^9-THC.

Results—Eight subjects (N=8) accurately discriminated Δ^9-THC and completed the study. The training dose of Δ^9-THC functioned as a discriminative-stimulus and produced prototypical subject-rated drug effects. All of the drugs tested produced significant effects on the self-report questionnaires, but only Δ^9-THC substituted for the training dose.

Conclusion—These results suggest that the discriminative-stimulus effects of Δ^9-THC in humans are not directly mediated through central neurotransmitter systems acted upon by the drugs tested in this study.

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Keywords

drug-discrimination; subjective effects; Δ^9-THC; triazolam; hydromorphone; methylphenidate; marijuana; cannabis

Introduction

The central effects of cannabis (*Cannabis sativa or Cannabis indica*) appear to be mediated primarily through cannabinoid (CB) receptors of the endogenous cannabinoid system. Two CB-specific receptors have been identified (Matsuda et al., 1990; Munro et al., 1993), and there is evidence of at least one additional subtype (Breivogel et al., 2001; Hajos et al., 2001). The CB_1 receptor subtype is primarily located in the central nervous system (Pertwee, 1997). The CB_2 receptor subtype was initially thought to be located exclusively in the periphery, particularly in the immune system (Kaminski et al., 1992), although recent data have demonstrated the functional expression of CB_2 receptors in neurons and glial cells in the brain (Onaivi et al., 2006). Nonetheless, several lines of evidence indicate that the central effects of cannabis and cannabinoids can be attributed to their actions at CB_1 receptors. The in vivo potencies of various cannabinoid ligands have been correlated with their binding affinities at CB_1 receptors (Compton et al., 1993), the distribution of CB_1 receptors in the CNS corresponds well with the effects of cannabis and cannabinoid ligands in animals and humans (Breivogel and Childers, 1998) and CB_1-selective antagonists, such as SR 141716A block the effects of cannabinoids in animals (reviewed in Chaperon and Thiebot, 1999) and of cannabis in humans (Gorelick et al., 2006; Huestis et al., 2001, 2007). Many of the phytocannabinoids present in cannabis bind to CB_1 receptors and are biologically active, however, Δ^9-tetrahydrocannabinol (Δ^9-THC) is widely held as the primary active constituent of cannabis. In support of this notion, there appear to be only subtle differences in the clinical effects of Δ^9-THC and cannabis (Chait and Zacny, 1992; Hart et al., 2002; Wachtel et al., 2002).

Although the endocannabinoid system, especially the CB_1 receptor subtype, appears responsible for mediating the behavioral and physiological effects of cannabis and cannabinoid ligands such as Δ^9-THC, there is evidence that non-CB neurotransmitter systems are also involved. A principal function of cannabinoid receptors is the modulation of non-CB neurotransmitter release via retrograde signaling (Hashimotodani et al., 2007; Szabo and Schlicker, 2005), so it stands to reason that these other neurotransmitter systems might play a role in the effects of cannabinoids. A considerable amount of preclinical research has focused on the involvement of GABA and opioid systems, and to a lesser degree, dopamine systems, in the effects of cannabinoids. There is substantial overlap in the effects produced by cannabinoids and drugs acting at central GABA and opioid systems, and neuroanatomical, neurochemical and behavioral studies support a functional link between the endogenous cannabinoids and these systems. CB_1 receptors are co-localized with GABA receptors throughout the brain, CB_1 receptor activity modulates the release of GABA, both GABA and CB ligands have been shown to impair memory and motor control, alleviate anxiety, induce hypothermia, increase feeding behavior, function as reinforcers, partially share discriminative-stimulus effects, and there are GABA-CB interactions on these outcomes (e.g., Barrett et al., 1995; DeSousa et al, 1994; Ferraro et al., 2001; Freund, 2003; Freund et al., 2003; Frosini et al., 2004; Griffiths and Weerts, 1997; Justinova et al., 2005; Kirkham, 2005; Mailleux and Vanderhaeghen, 1992; Ohno et al., 1992; Pertwee et al., 1988, 1991; Pertwee and Greentree, 1988; Rahminiwati and Nishimura, 1999; Rawls et al., 2004; Romero et al., 1996; van den Pol, 2003; Varvel et al., 2005; Wiley et al., 1995; Wilson and Nicoll, 2001). Likewise, CB_1 receptors are co-localized with opioid receptors throughout the brain, CB_1 receptor activity modulates the release of endogenous opioids, and there are shared effects of, and interactions between, opioids and cannabinoids on hypothermia, hypotension, intestinal motility, motor control,

analgesia, reinforcement and reward, drug discrimination, and self reported effects (reviewed in Fattore et al., 2004; Maldonado and Valverde, 2003; Manzanares et al., 1999; Viganò et al., 2005; see also Haney, 2007; Justinova et al., 2004; Mendizábal et al., 2006; Solinas and Goldberg, 2005). Fewer studies have examined interactions between dopamine and cannabinoid systems, but notably, like all other drugs of abuse, cannabinoids elevate dopamine in the mesocorticolimbic dopamine system, which is thought to play a necessary role in its abuse potential (Chen et al., 1990; Leshner and Koob, 1999). Together, these studies indicate that GABA, opioid and dopamine systems are involved, to some degree, in the effects of cannabinoids; however, relatively few data have been collected in humans, so further research would be informative.

The aims of this study were to determine whether oral Δ^9-tetrahydrocannabinol (Δ^9-THC) could function as a discriminative stimulus in humans and to examine the substitution profile of triazolam, hydromorphone and methylphenidate in humans discriminating Δ^9-THC in an attempt to examine the potential involvement of $GABA_A$, μ-opioid and dopamine receptors in the interoceptive effects produced by Δ^9-THC. Drug-discrimination was chosen as the primary outcome variable because the data from this procedure are concordant with the actions of a drug at the receptor level (Holtzman and Locke, 1988). Substitution drug-discrimination procedures were used because drugs from several pharmacological classes could be tested in a single study. An extensive review of clinical studies that used drug-discrimination and subject report measures to examine the neuropharmacology of a range of drug classes (Kelly et al., 2003) indicated that drug-discrimination data conformed more closely to the results from in vitro and in vivo preclinical studies.

Methods

Subjects

Healthy, adult men and women with a history of cannabis use were recruited from the local community to participate in this experiment. All potential subjects completed demographic, drug-use history, medical history and personality questionnaires, as well as medical screens. In addition, because the commercially available preparation of Δ^9-THC (Marinol®) is suspended in sesame oil, subjects were screened for an allergy to sesame seeds and sesame oil. Individuals with current or past histories of Axis I psychiatric disorder, including substance dependence disorders (except nicotine), were excluded from participating. All subjects were in good health with no contraindications to the drugs to be administered in the protocol. The Institutional Review Board of the University of Kentucky Medical Center approved the study and the informed consent document. The study was conducted in accordance with the ethical standards of the 1964 Declaration of Helsinki. All subjects provided sober, written informed consent and the confidentiality of their personal information was maintained throughout.

Fifteen healthy adult subjects were enrolled. Two subjects withdrew because they did not like the drug effects and four subjects withdrew for reasons unrelated to the experimental protocol. Another subject completed the study, but this subject's discrimination performance on sessions in which the control conditions were administered during the test phase (see below) was not different from chance, suggesting that the subject was unable to accurately discriminate Δ^9-THC. Data from these subjects were not included in the analyses. Eight subjects (4 Caucasian males, 1 Caucasian/Middle-Eastern male, 1 Black male, 2 Caucasian females) completed this experiment and maintained accurate discrimination performance throughout the experiment.

Subjects ranged in age from 20 to 29 years (median = 22 years) and in education from 12 to 20 years (median = 16). Subjects ranged in weight from 65 to 120 kg (median = 73 kg). All subjects reported moderate cannabis use (range of 1–5 times/week; mean = 2.5). Subjects reported consuming 1 to 24 standard alcohol-containing beverages per week (mean = 11.0).

Four subjects reported occasional tobacco cigarette use. Other lifetime drug use included amphetamine, cocaine, benzodiazepines, hallucinogens and opioids. Opioid use was primarily reported as therapeutic, but all other drug use was recreational. The reported frequency of other drug use was low, typically not in the month prior to screening. Consistent with the self-reported drug use, urine samples for seven of the eight subjects were positive for Δ^9-THC prior to any experimental drug administration. In addition, one subject tested positive for cocaine during the medical screening and the first practice session; all subsequent urine samples were negative for cocaine.

General Procedures

Subjects were enrolled as outpatients at the General Clinical Research Center (GCRC) at the University of Kentucky Medical Center. Subjects completed two practice sessions to become familiarized with the behavioral measures and daily laboratory routine. Experimental drugs were not administered during these sessions. Subjects then completed between 31 and 36 (mean = 33.3) experimental sessions. Subjects were paid $40 per session to participate in this experiment and received additional performance-based payment as outlined below.

Subjects were informed that during their participation they would receive placebo, Δ^9-THC, methylphenidate, hydromorphone and triazolam, administered orally, but were blind to the dose and order of administration. They were told that the purpose of the study was to see how different drugs affect mood and behavior and whether people are able to detect the presence of a drug. Subjects were asked to abstain from illicit drug use other than cannabis for the duration of the experiment, and to avoid any over-the-counter medication without prior approval, with the exception of non-steroidal anti-inflammatory analgesics. In addition, subjects were also asked not ingest food or caffeine for 4 hours prior to each experimental session, or alcohol for 12 hours prior to and following each experimental session.

Experimental sessions were conducted daily Monday through Friday, and subjects participated in 1–5 sessions per week. Subjects arrived at the GCRC between 8:00 to 9:00 AM on the day of scheduled sessions. Individual subjects were tested separately in a standard hospital room. At the beginning of each session, subjects completed field-sobriety, breath (Alcolyzer, AK Solutions USA, Palisades Park, NJ) and urine tests to assess drug use (Integrated E-Z Split Cut, Acon Laboratories, San Diego, CA) and possible pregnancy (hCG Assay, Rapid Detect, Inc., Poteau, OK). Subjects then consumed a low-fat snack. All female urine samples were negative for hCG.

Drug-Discrimination Procedure

This experiment used well-established drug-discrimination procedures in which subjects learn to discriminate between a Drug condition (i.e., 25 mg Δ^9-THC) and a Not Drug condition (i.e., placebo) (e.g., Babalonis et al., 2008; Griffiths et al., 1990; Heishman and Henningfield, 1991; Lile et al., 2004, 2005a,b, 2006, 2007; Stoops et al., 2005, 2006; Rush et al., 2000, 2003). We chose to use a Drug versus Not Drug discrimination because instructing subjects to discriminate these conditions have yielded results that are more consistent with the pharmacology of drugs compared to studies that instruct subjects to discriminate between Drug A and Drug B (e.g., active versus placebo) as the training conditions. (e.g., Preston and Bigelow, 2000; Preston et al., 1992).

The experiment was conducted in three phases, presented in fixed order.

Sampling Phase—During two sampling sessions, subjects ingested three capsules that contained a total of 25 mg Δ^9-THC. Δ^9-THC was identified by letter code (e.g., Drug X; a unique letter code used for each subject), but the subjects were not explicitly informed of the

contents of the capsules. Subjects were told that the capsules they were receiving were Drug X, and that they should pay attention to the effects of Drug X because in future sessions they can earn money by correctly identifying whether or not they have received Drug X.

Control Phase—The control phase was conducted to determine whether subjects could discriminate 25 mg Δ^9-THC from placebo. During this phase, subjects ingested capsules under double-blind conditions. The subjects were instructed that they must decide whether they have received Drug X or Not Drug X and that they can change their mind throughout the experimental session based on what they think at the time. The subjects were also told that if they think they have received Drug X they can earn money by responding that they have received Drug X or if they think they did not receive Drug X or if they received a drug that has effects different from Drug X, they can earn money by responding that they received Not Drug X. Finally, the subjects were instructed that at the end of some experimental sessions (i.e., control sessions), a computer screen would inform them as to whether Drug X or Not Drug X was administered and that during these sessions earnings will be determined based on correct responding on the drug-discrimination task (see below), but that during other sessions the drug condition would not be disclosed (i.e., test sessions, see below) and that earnings during these sessions will be based on the average of their earnings from control sessions. These instructions were also used during the test phase described below. The criterion for having acquired the discrimination was ≥ 80% correct responding on the drug-discrimination task during the final 5-h assessment for four consecutive sessions. The order of drug administration was random except that each subject received each training condition, 25 mg Δ^9-THC and placebo, at least twice every four sessions. If a subject did not meet the control criteria within 12 sessions, they were dismissed from the study.

Test Phase—Drug doses tested during the test phase included Δ^9-THC (5, 7.5, 15 and 25 mg) methylphenidate (5, 10, 20 and 30 mg), hydromorphone (0.75, 1.5, 3 and 4.5 mg), triazolam (0.0625, 0.125, 0.25 and 0.375 mg) and placebo. For comparison, dose ranges for the clinical application of these drugs, as listed in the Physician's Desk Reference (2004), are provided. The acute recommended dose range of Δ^9-THC as an anti-emetic in adults is 2.5–10 mg, and for appetite stimulation is 5–15 mg/m^2 (a means of dosing based on surface area instead of body weight; approximately 9–27 mg in a 70 kg person). The acute recommended dose range of methylphenidate for the treatment of attention deficit and hyperactivity disorder in adults is 20–30 mg. The acute recommended dose range of hydromorphone for the alleviation of pain in adults is 2–4 mg. The acute recommended dose range of triazolam for the treatment of insomnia is 0.125–0.5 mg. Each drug dose was administered once. The order of drug administration was random during this phase except that an active drug dose was never administered on more than three consecutive sessions.

Control sessions (i.e., 25 mg Δ^9-THC or placebo) were also included in the test phase to monitor drug-discrimination performance, and comprised approximately 32% of sessions during the test phase. If a subject responded incorrectly on a control session (i.e., < 80% correct at the final, 5-h assessment), additional control sessions were scheduled. These additional control sessions continued until the subject accurately identified both of the training conditions once each on consecutive sessions. Placebo and 25 mg Δ^9-THC were also included as test conditions (i.e., no feedback regarding drug-discrimination performance).

Outcome measures

In addition to drug-discrimination, several other measures were collected, including self-report questionnaires, performance tasks, physiological assessments, and a measure of drug reinforcement to more fully characterize the degree to which the behavioral and physiological effects of Δ^9-THC overlap with drugs acting at these various neurotransmitter systems. Only

a subset of the self-report questionnaire results will be presented to demonstrate that the dose range of each drug was biologically active and produced interoceptive effects.

The drug-discrimination task was completed 3, 4 and 5 h after drug administration; this abbreviated data collection schedule was used for this task based on pilot data from two subjects (data not shown) indicating that the onset of the discriminative-stimulus effects of Δ^9-THC did not emerge for 3–4 h after drug administration. Self-reported drug-effect data were collected immediately prior to drug administration, and 1, 2, 3, 4 and 5 h after drug administration.

Drug-Discrimination Task—A point-distribution drug-discrimination task (e.g., Lile et al., 2007) was used to assess the discriminative-stimulus effects of the various drug conditions. Two circles labeled Drug X and Not Drug X were displayed on the computer screen, each associated with a training dose condition. Counters were displayed directly below the circles. Mouse button presses increased the counter associated with the circle where the cursor was located according to a fixed-interval 1-sec schedule. The cursor could be moved between the circles without any consequence for the fixed-interval schedule (i.e., no change-over-delay). Up to 60 points could be allocated across the two options. During control sessions, points accumulated on the correct option were exchangeable for money at a rate of $0.24/point. Thus, subjects were able to earn a maximum of approximately $40.00/session on this task. The dependent variable for this task was the percent responding on the Drug X circle (i.e., drug-appropriate responding) at the final 5-h time point.

Subject-Rated Drug-Effect Questionnaires—Visual Analog Scale (VAS). Subjects rated 20 items (e.g., I feel: a good drug effect, high, shaky or jittery) presented individually on the computer by marking a 100-unit line anchored on the extremes by “Not At All” and “Extremely”.

Profile of Mood States (POMS). This 72-item adjective rating scale yields scores on eight mood clusters (e.g., Fatigue). Subjects rate each item by selecting one of five response options: “Not at all,” “A little bit”, “Moderately”, “Quite a bit” and “Extremely.” This version of the POMS consisted of the original 65-item experimental version of the POMS [i.e., described and validated by McNair et al. (1971)] plus an additional seven items (e.g., Fischman et al., 1990).

Drug Administration

All drug conditions were administered in a double-blind fashion. During each experimental session, subjects ingested three capsules with water. Methylphenidate (Mallinckrodt, St. Louis, MO), hydromorphone (Ethex Pharmaceuticals, St. Louis, MO) and triazolam (Roxane Labs, Columbus, OH) were prepared by encapsulating commercially available generic tablets in an opaque green size 00 capsule. Δ^9-THC capsules contained Marinol® (Solvay Pharmaceuticals, Marietta, GA) capsules, which consist of dronabinol (i.e., synthetic Δ^9-THC) in sesame oil. Cornstarch was used to fill the remainder of all capsules. Placebo capsules contained only cornstarch. Capsules were prepared by the University of Kentucky Medical Center Investigational Drug Service Pharmacy.

Data Analyses

Drug-discrimination data for each drug were analyzed separately as the percentage of drug-appropriate responding at the final, 5-h time point using one-factor, repeated-measure analysis of variance (ANOVA; JMP, SAS Institute Inc., Cary, NC) with Dose (Placebo, Dose 1, Dose 2, Dose 3 and Dose 4) as the factor. For the 25 mg Δ^9-THC and placebo conditions, data were averaged across the sessions in which these conditions were presented during the test phase. Raw data from the self-reported drug-effect questionnaires were analyzed for each drug as the

peak-effect (i.e., the mean of the maximum value observed for each subject 1–5 hr after drug administration) using one-factor, repeated-measure ANOVA. For all measures, effects were considered significant for $p \leq 0.05$. If the main effect of Dose attained statistical significance, planned comparisons using contrast statements were used to compare active drug doses to placebo.

Results

Drug-discrimination task

The 8 subjects met the discrimination criterion in an average of 5.1 sessions (range = 4–8). During the final four sessions of the control phase, subjects reported an average of 0.0 (SEM = 0.0) percent Δ^9-THC-appropriate responding on the drug-discrimination task during placebo sessions and 100.0 (SEM = 0.0) percent drug-appropriate responding during sessions when the training dose of Δ^9-THC (i.e., 25 mg) was administered.

The one-factor, repeated-measure ANOVA that included placebo and the 4 active drug doses of Δ^9-THC revealed a significant effect of Dose for percentage of Δ^9-THC-appropriate responding ($F_{4,28} = 8.3$, $p \leq 0.001$). When placebo and the training dose of Δ^9-THC were administered during the test phase, they occasioned an average of 3.4 (SEM = 3.4) and 92.7 (SEM = 5.1) percent Δ^9-THC-appropriate responding, respectively. The 7.5 and 15 mg doses of Δ^9-THC each occasioned 62.5 (SEM = 18.3) percent Δ^9-THC-appropriate responding, which, like the 25 mg dose of Δ^9-THC, were significantly greater than placebo (Figure 1).

The ANOVAs conducted on the point-distribution task data for the remaining drugs were not significant, indicating that no dose of any of the other drugs tested increased Δ^9-THC-appropriate responding above placebo levels.

Self-Reported Drug-Effect Questionnaires

Results from selected items from the self-reported drug-effect questionnaires chosen to illustrate the varying profile of the subject-rated effects across drugs and to demonstrate that all of the drugs tested produced interoceptive effects are presented in Figure 2. Δ^9-THC, triazolam, hydromorphone and methylphenidate significantly increased ratings on "positive" items from the self-reported drug-effect questionnaires. For example, the 7.5, 15 and 25 mg doses of Δ^9-THC, the 0.375 mg dose of triazolam and the 4.5 mg dose of hydromorphone significantly increased peak ratings on the item Good Drug Effects ($F's_{4,28} = 3.1–3.3$, $p's \leq 0.05$) from the VAS. Increased peak ratings on other questionnaire items were unique to a particular drug. For instance, only the 7.5, 15 and 25 mg doses of Δ^9-THC only significantly increased ratings on the item High ($F_{4,28} = 3.3$, $p \leq 0.05$) from the VAS. Triazolam alone increased ratings on the Total Fatigue scale from the POMS at the 0.375 mg dose ($F_{4,28} = 2.6$, $p \leq 0.05$). Finally, only methylphenidate increased ratings of Shaky or Jittery from the VAS at the 30 mg dose ($F_{4,28} = 3.6$, $p \leq 0.01$).

Discussion

One goal of the present study was to examine whether oral Δ^9-THC would function as a discriminative-stimulus in humans. In one previous study, human subjects learned to discriminate smoked cannabis containing an active concentration of Δ^9-THC (2.7%) from placebo cannabis (Chait et al., 1988). In that study, however, it was reported that some subjects based their discrimination responding, at least in part, on the taste or harshness of the cannabis cigarette. Oral Δ^9-THC was used in the present study to eliminate the external cues and/or expectations associated with smoked cannabis. Although smoked cannabis is the route of administration typically used in the natural environment, the present results with oral Δ^9-THC

are directly applicable to smoked cannabis for at least two reasons. First, it is widely accepted that Δ^9-THC is the primary active constituent of cannabis. Second, previous research that directly compared smoked cannabis with oral Δ^9-THC demonstrated that although the time course of the drug effects differed, the drug effect profile on behavioral and physiological measures was similar (Chait and Zacny, 1992; Hart et al., 2002; Wachtel et al., 2002).

Δ^9-THC functioned as a discriminative-stimulus and dose dependently increased drug-appropriate responding. Subjects learned to discriminate 25 mg Δ^9-THC from placebo within 8 control sessions. In general, discriminative control of behavior was maintained throughout the test phase. Six of the eight subjects correctly identified placebo or 25 mg Δ^9-THC every time a training condition was presented during the test phase. One subject inaccurately identified the active training condition (i.e., 25 mg Δ^9-THC) during the test phase on two separate occasions. In both cases, each of the training conditions was administered once during the next two consecutive sessions, and the subject correctly identified the training conditions in these additional sessions. The other subject who inaccurately identified the training conditions during the test phase was unable to attend experimental sessions for approximately 2 weeks during this phase for personal reasons, and required five additional presentations of the training condition to re-acquire the discrimination upon return. When a 5-fold range of Δ^9-THC doses were administered during the test phase, the lowest dose tested, 5 mg, engendered an average of 12.5% drug-appropriate responding, which consisted of 6 subjects reporting 0%, and 2 subjects reporting 100%, drug-appropriate responding. The same subjects also reported that the 7.5 and 15 mg doses of Δ^9-THC shared discriminative-stimulus effects with the training dose. The 7.5 and 15 mg doses of Δ^9-THC occasioned an average of 62.5% drug-appropriate responding, which was the result of 5 of 8 subjects allocating 100% of their responses to the drug-appropriate option and 3 of 8 allocating 0% of their responses to the drug-appropriate option. For 6 of the 8 subjects, the percent drug-appropriate responding was consistent across these two doses.

Because pilot data from two subjects (data not shown) indicated that the onset of the discriminative-stimulus effects of Δ^9-THC did not emerge for 3–4 h after drug administration, the drug-discrimination task was only presented 3, 4 and 5 h after drug administration in an effort to minimize random responding or guessing during the early time points when subjects would be unable to discern the interoceptive cues of Δ^9-THC. Those pilot data are consistent with the data shown here. When 25 mg Δ^9-THC was administered, subjects occasionally reported 0–50% drug-appropriate responding at the 3-h presentation of the drug-discrimination task, but allocated responding to the drug-appropriate option at the 4- and 5-h assessments. Likewise, the self-reported drug effect and physiological data for Δ^9-THC peaked at 3–4 h post-drug administration. It is possible that triazolam, hydromorphone or methylphenidate did not substitute for Δ^9-THC because the peak effects for these other drugs typically occurred an hour earlier (i.e., 2–3 h; data not shown). However, despite observable self-reported effects for these drugs at the 3-h time point, less than 34% drug-appropriate responding was engendered by any of the doses of triazolam, hydromorphone or methylphenidate at the earlier time points. In addition, we have found in previous studies that once subjects decide that the drug they received could be characterized as Drug or Not Drug, they distribute 100% of their responses to the same option for the remainder of the session, even once the acute effects of the drug have dissipated (e.g., Lile et al., 2006).

The second goal of this study was to examine the potential involvement of $GABA_A$, μ-opioid and dopamine receptors in the interoceptive effects produced by Δ^9-THC. With the exception of Δ^9-THC, none of the drugs tested shared discriminative-stimulus effects with the training dose of Δ^9-THC, despite the fact that they all produced self-reported effects. In drug-discrimination studies in animals, partial substitution for the discriminative-stimulus effects of Δ^9-THC was observed following administration of the $GABA_A$ positive modulators

diazepam, phenobarbital and pentobarbital (Barrett et al., 1995; Browne and Weissman, 1981; Järbe and Hiltunen, 1988; Mokler et al., 1986; Wiley et al., 1995, Wiley and Martin, 1999). These data indicate that there is an overlap in the interoceptive effects produced by $GABA_A$ positive modulators and cannabinoids, particularly since drugs from other pharmacological classes generally fail to engender Δ^9-THC-like discriminative-stimulus effects (Barrett et al., 1995; Browne and Weissman, 1981; Wiley et al., 1995). In the present study however, behaviorally active doses of triazolam did not occasion Δ^9-THC-appropriate responding. Worth noting is that when multiple $GABA_A$ positive modulators were tested under the same experimental conditions, diazepam engendered more Δ^9-THC-appropriate responding than chlordiazepoxide and midazolam (Barrett et al., 1995), suggesting that of the benzodiazepines tested, diazepam in particular produces an interoceptive cue similar to Δ^9-THC. Worth noting is that Δ^9-THC potentiated the discriminative-stimulus effects of pentobarbital when given in combination (Järbe and Ohlin, 1979; Järbe et al., 1975). Fewer research efforts have been aimed at evaluating μ-opioid agonists in animals trained to discriminate Δ^9-THC, although the available data from substitution studies indicate that opioids engender only low levels of drug-appropriate responding (Browne and Weissman, 1981; Järbe et al., 1988,Järbe et al., 2006; McMahon, 2006; Wiley et al., 1995). For example, in rats trained to discriminate Δ^9-THC, the μ-opioid agonists heroin or morphine did not engender drug-appropriate responding; however, when given in combination, these μ-opioid agonists shifted the Δ^9-THC generalization curve leftward (Solinas et al., 2004; Solinas and Goldberg, 2005). In the initial study, it was also demonstrated that Δ^9-THC elevated the endogenous μ-opioid agonist β-endorphin in the ventral tegmental area (VTA), and microinjections of β-endorphin into the VTA enhanced the discriminative-stimulus effects of non-discriminable doses of Δ^9-THC, but had no effect when administered alone. Even fewer studies have tested dopaminergic drugs in animals discriminating Δ^9-THC, but the available data have demonstrated that these drugs do not engender Δ^9-THC-like discriminative-stimulus effects (Bueno et al., 1976; Järbe et al., 2006; McMahon, 2006). Taken together with the present findings, it appears that the discriminative-stimulus effects of Δ^9-THC are not directly mediated by the $GABA_A$, μ-opioid, or dopamine receptors acted upon by the drugs tested. However, it appears that ligands at the $GABA_A$ and μ-opioid receptor subtypes can modulate the discriminative-stimulus effects of Δ^9-THC and that drug-discrimination procedures using drug combinations might be more sensitive to cannabinoid interactions with other neurotransmitter systems compared to substitution studies.

One of the limitations of the present study was a lack of a positive control. At the time this study was initiated, Δ^9-THC was the only cannabinoid agonist approved by the US Food and Drug Administration. However, the cannabinoid agonist nabilone (Cesamet®) was recently approved for the treatment of nausea and vomiting associated with cancer chemotherapy in patients who have failed to respond adequately to conventional anti-emetic treatments and is now commercially available (Cesamet® product information, 2006). Preliminary data from an ongoing study indicate that nabilone shares discriminative-stimulus effects with Δ^9-THC and would therefore be a useful positive control in future research (Lile et al., unpublished data). Worth noting is that interoceptive effects of nabilone were apparent at the 2–3 h following administration, demonstrating that a cannabinoid with a more rapid onset of action compared to Δ^9-THC engendered drug-appropriate responding, which suggests that the lack of substitution by triazolam, hydromorphone and methylphenidate is not the result of the Δ^9-THC discrimination being based on temporal cues.

The data generated by the present study established the procedures to investigate the discriminative-stimulus effects of Δ^9-THC in human subjects, which should prove useful for future research. For example, data from drug-discrimination studies with Δ^9-THC could provide important information about the neurobiological mechanisms underlying the effects of cannabinoids in humans. In addition to providing important basic science information, data

from those studies could also reveal potential targets other than cannabinoid systems for medications development to manage cannabis-use disorders, and similarly, drug-discrimination studies with Δ^9-THC could be used to screen potential medications. The results of this study also provide valuable groundwork for subsequent studies to examine the neuropharmacology of the effects of cannabinoids. Although the drug substitutions tested here did not reveal potential interactions with other neurotransmitter systems, the preclinical studies discussed above suggest that drug combination studies using these methods might be more sensitive, and support the use of these procedures for future research on the neuropharmacology of cannabinoids.

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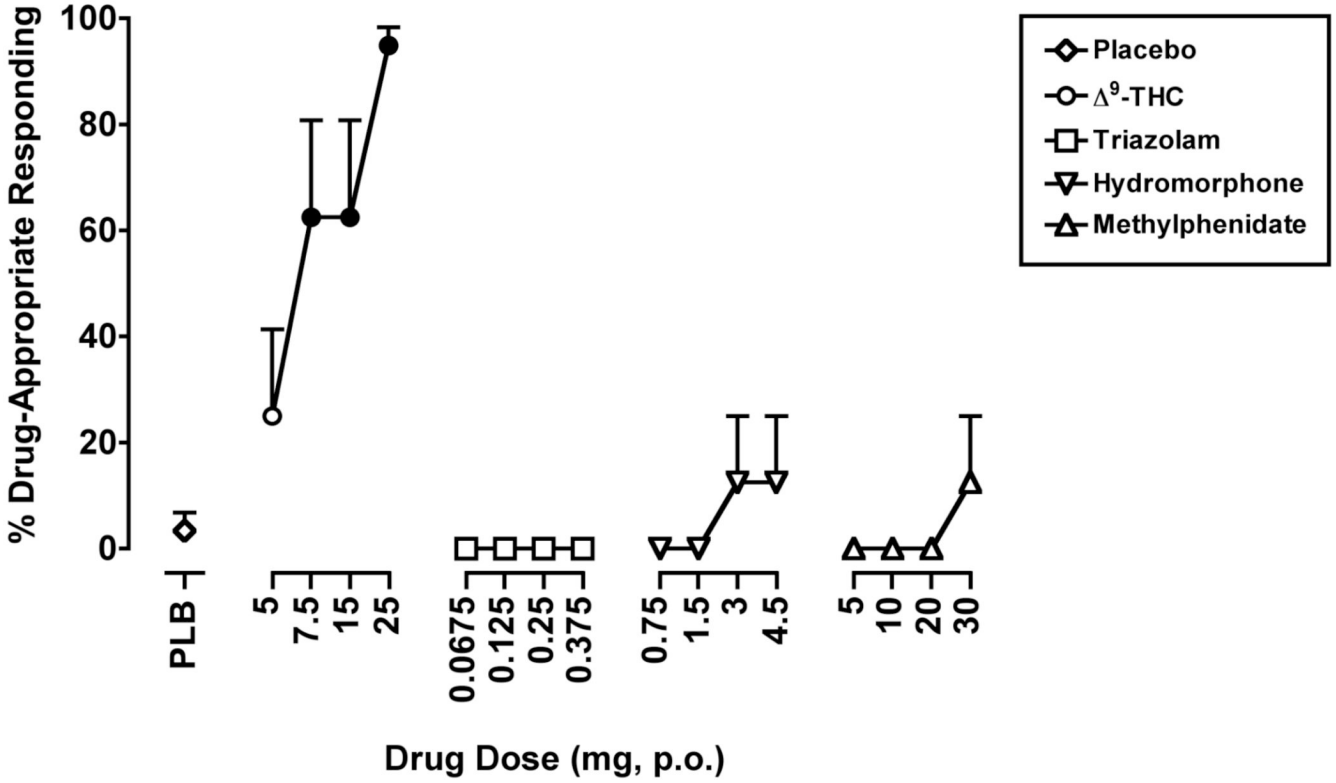

Figure 1.

The effects of Δ^9-THC, triazolam, hydromorphone and methylphenidate on Δ^9-THC-appropriate responding on the drug-discrimination task. Filled symbols indicate values that are significantly different from placebo. The x-axis represents the drug dose in mg; PLB denotes placebo. Data points show means of 8 subjects. Uni-directional brackets indicate 1 SEM.

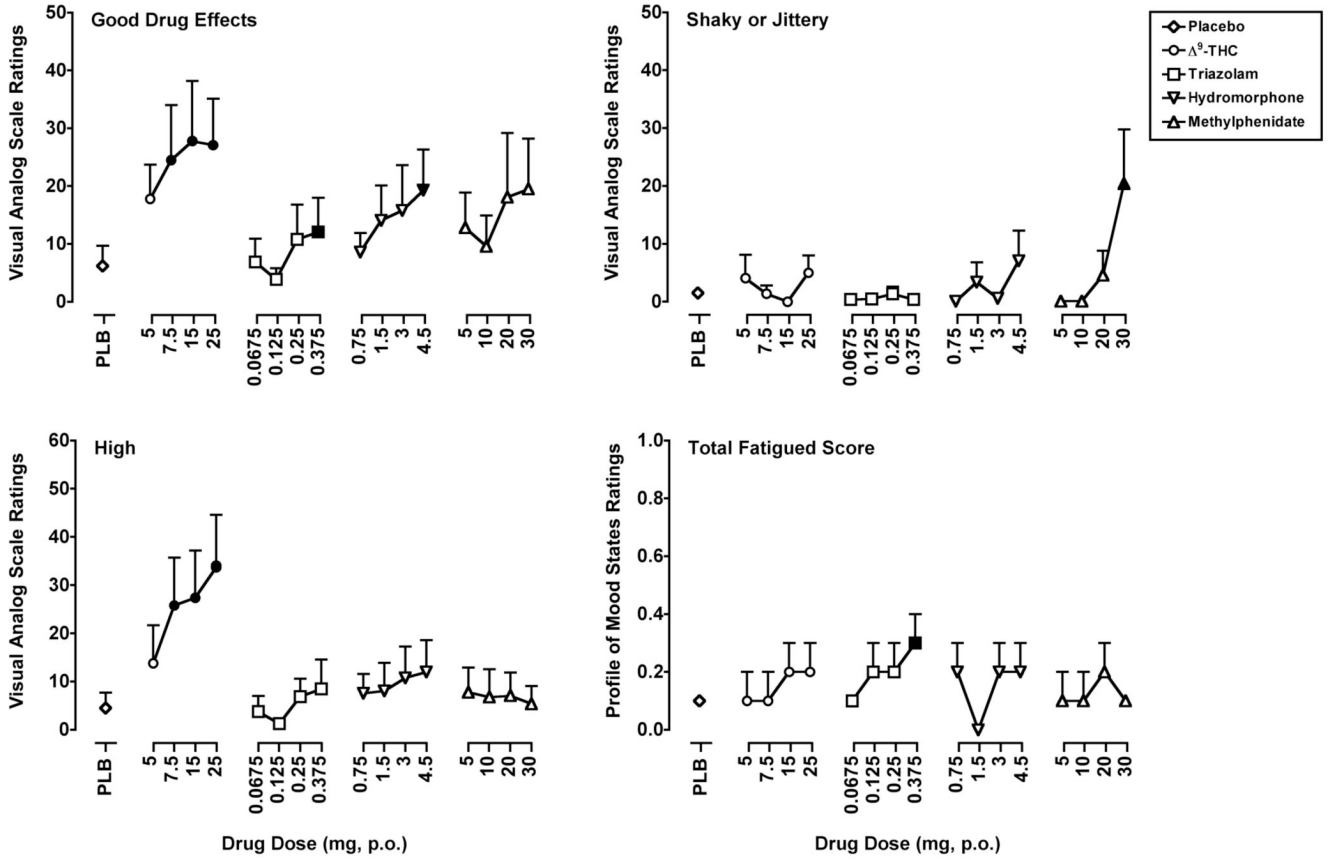

Figure 2.

Peak ratings for Δ^9-THC, triazolam, hydromorphone and methylphenidate on the Visual Analog Scale items Good Drug Effects, Shaky/Jittery and High and on the Profile of Mood States Total Fatigue scale. All other details are as in Figure 1.