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Separate and combined effects of the GABA reuptake inhibitor tiagabine and Δ^9 -THC in humans discriminating Δ^9 -THC

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Abstract

Background—The involvement of non-cannabinoid neurotransmitter systems in the abuse-related behavioral effects of cannabis has not been well characterized in humans. GABAergic drugs have overlapping effects with cannabis and Δ^9 -tetrahydrocannabinol (Δ^9 -THC) on certain behavioral measures, but those measures lack the specificity to draw conclusions regarding the involvement of GABA in cannabinoid effects. The aim of this study was to assess the separate and combined effects of the GABA reuptake inhibitor tiagabine and Δ^9 -THC using more pharmacologically specific drug-discrimination procedures.

Methods—Eight cannabis users learned to discriminate 30 mg oral Δ^9 -THC from placebo and then received tiagabine (6 and 12 mg), Δ^9 -THC (5, 15 and 30 mg) and placebo, alone and in combination. Self-report, task performance and physiological measures were also collected.

Results— Δ^9 -THC produced subjective effects typically associated with cannabinoids (e.g., High, Stoned, Like Drug), elevated heart rate and impaired rate and accuracy on psychomotor performance tasks. The higher tiagabine dose substituted for the Δ^9 -THC discriminative stimulus and engendered subjective and performance-impairing effects that overlapped with those of Δ^9 -THC when administered alone. In combination, tiagabine shifted the discriminative-stimulus effects of Δ^9 -THC leftward/upward and enhanced Δ^9 -THC effects on other outcomes.

Conclusions—These results indicate that GABA is involved in the clinical effects of Δ^9 -THC, and by extension, cannabis. Future studies should test selective GABAergic compounds to determine which receptor subtype(s) are responsible for the effects observed when combined with cannabinoids.

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Contributors

Drs. Lile, Kelly and Hays designed the study. Dr. Lile wrote the protocol; managed literature searches and summaries of previous related work; undertook the statistical analysis and graphical representation of the data; and wrote the first draft of the manuscript. Dr. Hays provided medical management and oversight. All authors contributed to and have approved the final manuscript.

Conflict of Interest

There are no conflicts of interest to declare.

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Keywords

drug-discrimination; marijuana; subjective effects; repeated acquisition task; digit-symbol-substitution task; cardiovascular

1. Introduction

The central effects of cannabis (*Cannabis sativa*, *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). The CB₁ receptor subtype is primarily located in the central nervous system, whereas the CB₂ receptor subtype is found mainly in the periphery (Pertwee, 1997; but see Onaivi, 2006). Several lines of evidence indicate that the central effects of cannabis and cannabinoids can be attributed to their actions at CB₁ receptors. First, the *in vivo* potencies of Δ^9 -THC and other cannabinoid ligands are correlated with their binding affinity at CB₁ receptors (Compton et al., 1993). Second, the distribution of CB₁ receptors in the central nervous system corresponds with the profile of Δ^9 -THC effects (Breivogel and Childers, 1998). Third, there is greater overlap in the behavioral effects of Δ^9 -THC with synthetic CB₁-selective agonists than CB₂-selective agonists (Järbe et al., 2006a; McMahon, 2006; Valenzano et al., 2005). Finally, CB₁-selective, but not CB₂-selective, antagonists block the centrally mediated effects of Δ^9 -THC and cannabis (Compton et al., 1996; Järbe et al., 2006b; Huestis et al., 2001, 2007; Zuurman et al., 2010).

Although the endocannabinoid system, especially the CB₁ receptor subtype, is primarily responsible for the behavioral and physiological effects of cannabis and CB ligands such as Δ^9 -THC, there is evidence that non-CB neurotransmitter systems are also involved. A principal function of CB 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 other neurotransmitter systems might play a role in the effects of cannabinoids. In particular, neuroanatomical and neurochemical studies support a functional link between endogenous cannabinoid and GABA systems. CB₁ receptors are co-localized with GABA receptors in regions throughout the brain, including the hippocampus, cortex, basolateral and cortical amygdaloid nuclei, striatum, cerebellum and the majority of hypothalamic nuclei (Freund, 2003; Hohmann and Herkenham, 2000; Mailleux and Vanderhaeghen, 1992; Westlake et al., 1994). Additionally, CB₁ receptor activity modulates synaptic GABA levels (Antonelli et al., 2009; Engler et al., 2006; Ohno-Shosaku et al., 2001; Wilson and Nicoll, 2001). For example, GABAergic transmission is enhanced by reducing GABA reuptake via stimulation of presynaptic cannabinoid receptors in certain GABA neurons (Banerjee et al., 1975; Maneuf et al., 1996; Romero et al., 1998) and also possibly by direct interactions with the GABA transporter (Coull et al., 1997).

Preclinical research that has directly compared or combined CB agonists and drugs that elevate central GABA via blockade of the reuptake transporter has demonstrated an overlap in some of their central effects. For instance, GABA reuptake inhibition by NO-328 (i.e., tiagabine) augmented the catalepsy (but not the antinociception or hypothermia) produced by Δ^9 -THC in rodents, (Pertwee et al., 1988, 1991). Likewise, individual studies have shown that under certain conditions, CB agonists and GABA transporter blockers are anxiolytic (Moreira and Wotjak, 2010; Schmitt and Hiemke, 1999), induce hypothermia (Frosini et al., 2004; Wenger and Moldrich, 2002), impair memory processes and motor activity (Castellano et al., 2003; Sañudo-Peña et al., 2000; Schmitt and Hiemke, 2002; Suzdak et al., 1992), and produce antinociception (Hohmann, 2002; Laughlin et al., 2002). Furthermore, in

separate clinical studies, CB agonists and tiagabine have each been shown to alleviate pain and anxiety (Bestard and Toth, 2011; Glass et al., 1980; Karst et al., 2010; Nakano et al., 1978; Schwartz and Nihalani, 2006; Zwanzger et al., 2003).

Taken together, these studies indicate that GABAergic drugs have overlapping effects with cannabis and Δ^9 -THC on certain behavioral measures, but those measures lack the specificity to draw conclusions regarding the involvement of GABA in cannabinoid effects. The aim of this study was to assess the separate and combined effects of the GABA reuptake inhibitor tiagabine and Δ^9 -THC using drug-discrimination procedures, which are concordant with the central actions of a drug at the receptor level, and therefore have greater pharmacological specificity (Holtzman and Locke, 1988). For example, in previous studies in which human subjects learned to discriminate Δ^9 -THC, the cannabinoid agonist nabilone, but not methylphenidate, triazolam or hydromorphone, occasioned drug-appropriate responding, whereas each of these drugs increased ratings on positive self-reported drug-effect questionnaire items (Lile et al., 2009, 2010).

2. Methods

2.1. Subjects

Adult men and women with a history of cannabis use were recruited from the local community. Potential subjects completed demographic, drug-use history and medical history questionnaires, as well as medical screens. Individuals with current or past histories of Axis I or DSM-IV psychiatric disorder, including substance dependence disorders other than nicotine, were excluded from participating. Substance abuse was not an exclusion criteria. Six of the subjects met at least one, but fewer than three of the DSM-IV criteria for cannabis dependence, and none met nicotine dependence criteria. The Institutional Review Board of the University of Kentucky Medical Center approved the study and the informed consent document.

Thirteen subjects were screened for participation, but two were discharged before completing test sessions (one moved from the area, and one became pregnant). Of the 11 subjects beginning the study, one was discharged for inconsistent attendance and 2 withdrew (one due to undesirable side effects of oral Δ^9 -THC, and contact was lost with one). Eight subjects (4 white males, 1 Hispanic/Latino male, 2 white females and 1 Hispanic/Latino female) completed the experiment. They ranged in age from 18 to 30 years (median = 22 years), in education from 11 to 18 years (median = 15), and in weight from 66 to 110 kg (median = 75 kg).

All subjects reported cannabis use (range of 1 to 7 days/week; mean = 4.5). At the time of screening, subjects reported consuming 0 to 5 standard alcohol-containing beverages per week (mean = 1.8). Three subjects reported using 1 to 8 tobacco cigarettes per day. Other lifetime non-medical drug use included benzodiazepines (1 subject, not in the month prior to screening), hallucinogens (four subjects, none reporting use in the month prior to screening), opioids (three subjects, with one reporting medical use as prescribed by a physician in the month prior to screening) and stimulants (cocaine: one subject, methylphenidate: two subjects, amphetamines: one subject and phenylethylamines: one subject, with methylphenidate being used on 3 occasions and amphetamine reported being used on a single occasion in the month prior to screening). All subjects provided a urine sample negative for substances other than cannabinoids prior to study initiation.

2.2. General Procedures

Subjects were enrolled as outpatients at the University of Kentucky General Clinical Research Center. They completed two drug-free practice sessions to become familiarized

with the procedures prior to completing between 22 and 26 (mean = 23.4) experimental sessions. Study participation lasted between 7 to 11 weeks (mean = 9).

Subjects were informed that they would receive placebo, Δ^9 -THC and tiagabine, alone or in combination, but were blind to the dose and order of administration. They were asked to abstain from illicit drugs other than cannabis for the duration of the experiment, and any drug use on the day of experimental sessions to avoid potentially unsafe drug interactions. They were also asked to avoid any over-the-counter medication, with the exception of non-steroidal anti-inflammatory analgesics. In addition, subjects were asked to refrain from food or caffeine intake for 4 hours prior to each experimental session, or alcohol for 12 hours prior to and following each experimental session. Subjects who smoked tobacco cigarettes were also asked to abstain from smoking the morning of each session, but were allowed to smoke a single tobacco cigarette upon arrival to the laboratory to avoid testing under conditions of nicotine withdrawal. They were not allowed to smoke again until the session had ended.

Experimental sessions were conducted at a fixed time, Monday through Friday, and lasted approximately 7.5 h; subjects participated in 1 to 5 sessions per week. At the beginning of each session, 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 pregnancy (hCG Assay, Rapid Detect, Inc., Poteau, OK) were conducted. Urine samples were negative for substances other than cannabis metabolites (i.e., 11-nor-9-carboxy- Δ^9 -THC) and hCG throughout the study, with the following exceptions. Urine toxicology screening for one subject was positive for methylenedioxymethamphetamine (MDMA); this subject was sent home but was permitted re-initiate study participation once a urine sample negative for all non-cannabinoid drugs was provided. At session intake, subjects also completed a modified version of the U.S. Department of Transportation Drug Evaluation and Classification Screening (walk and turn, timed one-leg balance or Romberg balance, time interval reproduction and the finger-to-nose tests; Toland and Green, 1991) and were observed by the research staff for signs of cannabis intoxication (e.g., bloodshot, glassy eyes); no cannabis intoxication was detected during intake throughout the study. Subjects were reassessed at the end of the session for possible intoxication and/or impairment using these procedures prior to release. In addition, subjects were required to report no further drug effects. If necessary, subjects were retained at the laboratory beyond the scheduled session time until residual drug effects dissipated.

Subjects consumed a low-fat snack approximately 20 minutes prior to drug administration. Because the peak plasma levels of Δ^9 -THC occur 2 to 4 h following administration (Hollister et al, 1981) whereas tiagabine concentrations peak 1 to 3 h after dosing (Gabitril® Product Information, 2009), tiagabine was administered 1 h after Δ^9 -THC so the peak levels of Δ^9 -THC and tiagabine occurred simultaneously. Data collection time points refer to Δ^9 -THC administration.

2.3. Drug-Discrimination Procedure

Well-established drug-discrimination procedures (e.g., Lile et al., 2009; Rush et al., 1998) were used to teach subjects to discriminate between a “Drug X” condition (i.e., 30 mg Δ^9 -THC) and a “Not Drug X” condition (i.e., placebo).

Sampling Phase—During two sampling sessions, subjects ingested four capsules that contained a total of 30 mg Δ^9 -THC. The capsules were identified by a letter code (e.g., Drug X; a unique letter code was used for each subject); subjects were not informed that the capsules contained Δ^9 -THC, but were instructed to associate drug effects with the letter code.

Control Phase—A control phase, lasting between 4 and 12 sessions, was conducted to determine whether subjects could discriminate 30 mg Δ^9 -THC from placebo. During this phase, subjects ingested capsules under double-blind conditions. The order of drug administration was random except that all subjects received each training condition, 30 mg Δ^9 -THC and placebo, at least twice every four sessions. Sessions were identical to the sampling phase, except subjects were not informed which drug condition (i.e., Drug X or Not Drug X) was administered until the end of the session. The criterion for having acquired the discrimination was $\geq 80\%$ correct responding on the drug-discrimination task during the final 6 h assessment for four consecutive sessions. If subjects did not meet the control criteria within 12 sessions, they would have been dismissed from the study. Six subjects correctly identified each of the two training conditions, 30 mg Δ^9 -THC and placebo, twice in the first four sessions. One subject required six sessions, and another subject required 8 sessions, to meet the discrimination criteria.

Test Phase—A final test phase, lasting at least 16 sessions, was conducted to test placebo, Δ^9 -THC (5, 15 and 30 mg) and tiagabine (6 and 12 mg), alone and in combination. Each drug dose and dose combination was administered once for a total of 12 sessions. The order of drug administration was random except that an active drug dose was never administered on more than three consecutive sessions, and the highest dose of Δ^9 -THC (30 mg) and tiagabine (12 mg) were not administered together before a lower dose combination was tested.

Four control sessions (i.e., 30 mg Δ^9 -THC or placebo) were also randomly included in the test phase to monitor drug-discrimination performance and provide feedback to subjects regarding their performance. If a subject responded incorrectly on a control session, additional control sessions were scheduled until the subject accurately identified both of the training conditions once each across consecutive sessions. Only one subject incorrectly identified control sessions during the test phase, resulting in the addition of four more control sessions (two each of the 30 mg Δ^9 -THC or placebo conditions) for that individual. Control sessions comprised an average of 26% of sessions during the test phase.

2.4. Outcome measures

Drug discrimination was the primary outcome measure, supplemented by self-report questionnaires, performance tasks and physiological assessments. Data were collected in fixed order, immediately prior to drug administration, and 1, 2, 3, 4, 5 and 6 h after Δ^9 -THC administration, with the following exceptions. The drug-discrimination task was completed at only the 3 to 6 h time points because of the slow onset (3 to 4 h) of the effects of Δ^9 -THC observed in our previous studies (Lile et al., 2009, 2010). A non-contingent Multiple-Choice Procedure was completed at the end of the 6-h assessment. Except for temperature assessments, data were collected on an Apple Macintosh computer (Apple Computer, Inc., Cupertino, CA).

Drug-Discrimination Task—Two circles labeled Drug X and Not Drug X and associated counters were displayed on a computer screen. Button presses increased the counter for a particular circle according to a fixed-interval 1-sec schedule for 60 s (no change-over delay). At the end of the final assessment, subjects were informed whether it was a control or a test session. During control sessions, points accumulated on the correct option were exchangeable for money at a rate of \$0.21/point. Thus, subjects were able to earn up to approximately \$50.00/session on this task. During test sessions, when drugs and/or doses other than the control conditions (i.e., 30 mg Δ^9 -THC or placebo) were administered, subjects earned the average from all previous sessions in which control conditions were tested. These monetary contingencies prompted subjects to acquire points on the counters

based on the presence (or absence) of the training drug cue at the time of task performance during both control and test sessions. The dependent variable for this task was the percent responding on the drug-appropriate option at the 6-h time point.

Subject-Rated Questionnaires: Visual Analog Scale (VAS) Subject-Rated Drug-Effect Questionnaire. Subjects rated 20 items (I feel: any drug effect, a bad drug effect, confused, dizzy, forgetful, a good drug effect, high, hungry, nauseated, restless, sedated, shaky or jittery, stimulated, stoned, suspicious, thirsty; I am seeing or hearing unusual things; I like the drug effect; I would pay for the drug; I would take this drug again) presented individually on the computer by marking a 100-unit line anchored on the extremes by “Not At All” and “Extremely”.

Multiple-Choice Procedure—This task provided an assessment of the monetary value of each dose condition (Griffiths et al., 1993). Subjects made a series of nine discrete choices between the drug dose received during that session and ascending amounts of money. The dollar value increased across the choices (\$0.10, 0.25, 0.50, 1.00, 2.00, 4.00, 8.00, 16.00 and 32.00). The dependent measure on the Multiple-Choice Procedure was the maximum dollar value at which subjects chose drug over money (i.e., “crossover point”). There were no contingencies associated with subject choices on this task.

Performance Tasks—These tasks were chosen because prior research has found them to be sensitive to the impairing effects of oral Δ^9 -THC (Hart et al., 2005; Kamien et al., 1994) and smoked cannabis (Heishman et al., 1989; Kelly et al., 1990, 1993; Wilson et al., 1994). Subjects did not receive additional compensation based on task performance.

Repeated Acquisition of Response Sequences Task (RA task)—During the initial acquisition component, subjects pressed 4 keys (1, 3, 7 and 9) on a numeric keypad to learn a new, randomly-determined 10-response sequence (a “chain”) for 180 s. When a correct key in the sequence was pressed, a “position” counter on the screen increased by 1. When the tenth and final key in the sequence was pressed, a “points” counter increased by one, and the position counter reset. A 60-s performance component of this task, in which the 10-response sequence remained the same across trials, followed the acquisition component. The primary dependent measures for this task were the number of chains completed (i.e., accuracy) and the total number of responses emitted (i.e., response rate).

Digit-Symbol-Substitution Test (DSST)—A modified version of the computerized DSST was used (McLeod et al., 1982). Briefly, subjects used a numeric keypad to enter the geometric pattern associated with one of nine patterns identified on a given trial for 90 s. The dependent measures were the number of patterns the subject entered correctly (i.e., trials correct; accuracy) and the total number of patterns entered (i.e., trials completed; response rate).

Time Reproduction Task—Four time periods, 3, 30, 60 and 180s were presented. Subjects responded to start a timer, and held down the response key until they believed that the interval had elapsed.

Physiological Indices: Heart Rate and Blood Pressure. Heart rate and blood pressure were recorded using an automated monitor (DINAMAP, Johnson and Johnson, Alexandria, TX).

Temperature—An infrared thermographic scanner (Derma-Temp, Exergen Corporation, Watertown, MA) was used to measure skin temperature on the tip of the index finger. An electronic thermometer was used to measure oral temperature.

2.5. Drug Administration

Doses of Δ^9 -THC were prepared by encapsulating commercially available capsules of Marinol® (Δ^9 -THC in sesame oil, Solvay Pharmaceuticals, Marietta, GA) in three opaque green size 00 capsules. Tiagabine was administered in one opaque blue/white size 0 capsule and contained commercially available Gabitril® (Cephalon, Inc., Frazer, PA). Cornstarch was used to fill the remainder of all capsules. Placebo capsules contained only cornstarch.

For reference, the acute recommended Δ^9 -THC dosing range in adults for appetite stimulation and the prevention of nausea and vomiting is 2.5 to 20 mg (Marinol® product information). The starting dose of tiagabine for epilepsy is 4 mg (Gabitril® product information). Important to note, however, is that other studies have administered acute doses of 12 to 24 mg tiagabine without reports of serious adverse events (e.g., Bialer, 1993; Zwangser et al., 2005). In an earlier version of the present protocol, 24 mg tiagabine alone was tested in a single subject, but the subject experienced sedation and confusion; consequently, the tiagabine doses were reduced to 6 and 12 mg.

2.6. Data Analyses

Data from the eight subjects who completed the study were analyzed statistically. Drug-discrimination data were analyzed as percent drug-appropriate responding using two-factor, repeated-measure analysis of variance (ANOVA; JMP, SAS Institute Inc., Cary, NC) with Δ^9 -THC and tiagabine as the factors. For the 30 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, performance tasks and physiological measures were analyzed for each drug as the peak-effect (i.e., the mean of the maximum or minimum value observed for each subject 1 to 6 h after drug administration) using two-factor, repeated-measure ANOVA. Crossover point data from the Multiple-Choice Procedure were first subjected to a square-root transformation because of violations in the assumptions of ANOVA (i.e., monetary increments across successive choices range from \$0.15 to \$16.00). For all measures, effects were considered significant for $p \leq 0.05$. If a main effect of Δ^9 -THC attained statistical significance, contrast statements were used to compare active drug doses to placebo; if a main effect of tiagabine, or an interaction of Δ^9 -THC and tiagabine, attained statistical significance, each dose of Δ^9 -THC alone was compared to that dose of Δ^9 -THC administered in combination with tiagabine.

3. Results

3.1. Drug-discrimination task

All subjects met the discrimination criterion, which took an average of 4.7 sessions (range = 4 to 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., 30 mg) was administered.

The two-factor, repeated-measure ANOVA revealed a significant interaction of Δ^9 -THC and tiagabine ($F_{6,42} = 5.3$, $p < 0.001$) for percentage of Δ^9 -THC-appropriate responding. The discriminative-stimulus effects of Δ^9 -THC, alone and in combination with tiagabine are shown in Figure 1. During the test phase, placebo and the training dose of Δ^9 -THC occasioned an average of 10.9 (SEM = 6.4) and 89.1 (SEM = 7.6) percent Δ^9 -THC-appropriate responding, respectively. Δ^9 -THC alone dose-dependently increased drug-appropriate responding on the drug-discrimination task. The 12 mg dose of tiagabine alone also significantly increased drug-appropriate responding, and significantly shifted the discriminative-stimulus effects of the 5 and 15 mg doses of Δ^9 -THC leftward/upward. The 6

mg tiagabine dose alone did not differ from placebo, but significantly enhanced the discriminative-stimulus effects of the 5 mg dose of Δ^9 -THC.

3.2. Subject Ratings

An interaction of Δ^9 -THC and tiagabine ($F_{6,42} = 2.3-3.6$, $p's \leq 0.05$) was detected for eight VAS items: Any Effect*, High*, Like Drug*, Sedated*, Bad Effects, Take Again, Hungry and Thirsty. The data from VAS items marked with an asterisk* are presented in Figure 2. For five VAS items, significant main effects of Δ^9 -THC ($F_{3,21} = 3.2-23.3$, $p's \leq 0.05$) and tiagabine ($F_{2,14} = 3.5-15.4$, $p's \leq 0.05$) were found: Good Effects, Pay For, Restless, Stoned, Forgetful. In general, both drugs increased ratings on these items alone, and the effects of the 5 mg or 5 and 10 mg doses of Δ^9 -THC were significantly enhanced by tiagabine in a dose-dependent manner. In addition, tiagabine alone increased ratings of Stimulated ($F_{2,14} = 5.0$, $p \leq 0.05$) and Dizzy ($F_{2,14} = 5.5$, $p \leq 0.01$).

3.3. Multiple-Choice Procedure

A significant interaction was observed for Δ^9 -THC and tiagabine ($F_{6,42} = 2.4$, $p \leq 0.05$) on the crossover point. Δ^9 -THC increased crossover point relative to placebo at the 15 and 30 mg doses. The 12 mg dose of tiagabine significantly increased crossover point alone, and relative to Δ^9 -THC alone at the 5 mg dose (data not shown).

3.4. Performance

Main effects of Δ^9 -THC ($F_{3,21} = 3.3$, $p \leq 0.05$) and tiagabine ($F_{2,14} = 7.5$, $p \leq 0.01$) were found for the number of chains completed on the acquisition component of the RA task. Likewise, main effects of Δ^9 -THC ($F_{3,21} = 3.3$, $p \leq 0.05$) and tiagabine ($F_{2,14} = 7.7$, $p \leq 0.01$) were found for the total number of responses emitted. For these outcomes, performance was impaired by all active doses of Δ^9 -THC. The 12 mg dose of tiagabine alone also reduced rate and accuracy relative to placebo and enhanced performance impairment at the 15 and 30 mg Δ^9 -THC doses. The effects of Δ^9 -THC and tiagabine on rate and accuracy on the acquisition component of the RA task are presented in Figure 3.

With respect to the performance component of the RA task, main effects of Δ^9 -THC ($F_{3,21} = 3.2$, $p \leq 0.05$) and tiagabine ($F_{2,14} = 8.5$, $p \leq 0.01$) were detected on the number of chains completed. Both Δ^9 -THC ($F_{3,21} = 3.1$, $p \leq 0.05$) and tiagabine ($F_{2,14} = 8.8$, $p \leq 0.01$) also significantly impacted the total number of responses emitted. For these outcomes, performance was impaired at the 15 and 30 mg doses of Δ^9 -THC and 12 mg dose of tiagabine. This tiagabine dose also enhanced performance impairment at the 30 mg Δ^9 -THC dose (data not shown).

ANOVA revealed significant main effects of Δ^9 -THC ($F_{3,21} = 3.8$, $p \leq 0.02$) and tiagabine ($F_{2,14} = 11.9$, $p \leq 0.001$) to reduce the number of correct trials on the DSST. A main effect of tiagabine ($F_{2,14} = 9.7$, $p \leq 0.01$) was also observed for DSST trials completed (trend for Δ^9 -THC, $p = 0.08$). The 12 mg dose of tiagabine further disrupted rate and accuracy in combination with the 5 and 15 mg Δ^9 -THC doses, as well as when it was administered alone (data not shown).

Tiagabine and Δ^9 -THC had inconsistent effects on the reproduction of time (data not shown). A main effect of tiagabine was observed on the Time Reproduction task for the 30-s ($F_{2,14} = 7.6$, $p \leq 0.01$), 60-s ($F_{2,14} = 7.7$, $p \leq 0.01$) and 180-s ($F_{2,14} = 6.3$, $p \leq 0.01$) intervals. Δ^9 -THC impacted performance on this task only at the 180-s interval ($F_{3,21} = 3.3$, $p \leq 0.05$). Administration of the 12 mg dose of tiagabine resulted in under-reproduction of the 30-s interval, and both tiagabine doses had this effect at the 180-s interval, regardless of Δ^9 -THC dose. Only the 15 mg dose of Δ^9 -THC alone resulted in the under-reproduction of

time, and only at the 180-s interval. No significant differences of tiagabine were identified when compared to placebo or doses of Δ^9 -THC alone on the 60-s interval. Neither Δ^9 -THC or tiagabine affected reproduction of the 3-s time interval.

3.5. Heart Rate, Blood Pressure and Temperature

Δ^9 -THC significantly elevated heart rate in a dose-dependent manner ($F_{3,21} = 15.1$, $p < 0.001$), but tiagabine had no effect alone or in combination with Δ^9 -THC (Figure 4). Blood pressure was not impacted by Δ^9 -THC or tiagabine (data not shown).

An interaction of tiagabine and Δ^9 -THC was found for index finger skin temperature ($F_{6,42} = 2.6$, $p \leq 0.05$), but did not appear to be a function of dose. Compared to placebo, the 5 mg Δ^9 -THC + 12 mg tiagabine dose and the 30 mg Δ^9 -THC dose alone decreased index finger skin temperature. In contrast, both active tiagabine doses increased index finger skin temperature when combined with 30 mg Δ^9 -THC (data not shown). Oral temperature was not influenced by Δ^9 -THC or tiagabine (data not shown).

4. Discussion

The aim of this study was to assess the separate and combined effects of the GABA reuptake inhibitor tiagabine and Δ^9 -THC using drug-discrimination procedures. Δ^9 -THC functioned as a discriminative stimulus and dose-dependently occasioned drug-appropriate responding, consistent with previous studies (Lile et al., 2009, 2010, 2011). The larger tiagabine dose alone occasioned Δ^9 -THC-appropriate responding, and when combined with Δ^9 -THC, both tiagabine doses significantly enhanced drug-appropriate responding, resulting in full substitution of the lowest dose of Δ^9 -THC. Similar potentiation was observed on the self-reported and performance measures. Heart rate was the only measure for which there was a significant effect of Δ^9 -THC but not tiagabine. The leftward/upward shift in the dose-effect curves across several cannabinoid-sensitive measures suggests the involvement of GABA in the behavioral effects of cannabinoids in humans.

4.1. Drug discrimination

The substitution of the higher tiagabine dose and potentiation of the discriminative-stimulus effects of Δ^9 -THC by concurrent administration with tiagabine is remarkably similar what was observed in our previous study in which the CB agonist nabilone was administered alone and in combination with Δ^9 -THC (Lile et al., 2011). In that study, both Δ^9 -THC and nabilone alone shared discriminative-stimulus effects with the training dose of Δ^9 -THC, increased crossover point on the Multiple-Choice Procedure, produced overlapping subject ratings and decreased skin temperature. In combination, nabilone significantly shifted the discriminative-stimulus effects of Δ^9 -THC leftward/upward and enhanced Δ^9 -THC effects on cannabinoid-sensitive measures. Worth noting is that a previous human study tested 4 and 8 mg of tiagabine in subjects discriminating oral cocaine (Lile et al., 2004), and did not find changes in the cocaine dose-effect curve when combined with tiagabine. The impact of tiagabine on the discriminative-stimulus effects of Δ^9 -THC, but not cocaine, suggest a role for GABA in the behavioral effects of cannabinoids rather than a general impact on drug-discrimination performance.

Also important to note is that the drug combination procedures used here appeared to permit greater sensitivity to detect the possible involvement of non-cannabinoid neurotransmitter systems in the effects of Δ^9 -THC compared to substitution procedures alone. Specifically, when combined with either active tiagabine dose, the discriminative-stimulus effects of Δ^9 -THC were significantly increased compared to that dose of Δ^9 -THC alone. In contrast, when tiagabine was administered alone, only the higher, 12-mg dose substituted for the Δ^9 -THC

discriminative stimulus. This finding is important because it suggests that low doses of GABAergic compounds could be used in future pharmacotherapeutic applications to modulate Δ^9 -THC effects, or low doses of each could be combined for therapeutic use, which might circumvent side effects of higher doses of the constituent drugs.

4.2. Abuse potential of tiagabine and tiagabine-cannabinoid combinations in cannabis users

In the present study, tiagabine significantly increased crossover point on a non-contingent Multiple-Choice Procedure. Tiagabine also increased ratings on “positive” items from the self-reported drug-effect questionnaire, such as High and Like Drug. These results contrast with previous studies that have administered tiagabine and failed to detect significant subjective effects suggestive of abuse potential in cocaine-dependent individuals (Lile et al., 2004; Sofuoglu et al., 2005a), tobacco smokers (Sofuoglu et al., 2005b), patients with epilepsy (Fritz et al., 2005) and healthy volunteers (Kastberg et al., 1998; Walsh et al., 2005). Consistent with the possibility that tiagabine might have increased abuse potential in cannabis users, this group appears to use sedative-type psychoactive drugs more frequently than non-cannabis users. Specifically, survey data indicate that the percentage of past-month cannabis users who report past-month use of prescription sedatives such as benzodiazepines is greater than the number of non-users reporting past-month prescription sedative use (5.6% versus 0.4%; SAMHSA, 2008). Several factors could account for the association between cannabis use and the use of sedative-type drugs, including a general predisposition to drug use stemming from environmental and/or genetic influences, enhanced access to and socialization within illicit drug-use and drug-trade networks, or neurobiological changes that could occur with long-term cannabis use (e.g., Hall and Lynskey, 2005; Rubino and Parolaro, 2008; Vanyukov et al., 2003). Worth noting is that there appeared to be a ceiling on the combined effects of tiagabine and Δ^9 -THC on the self-reported items and crossover point on the Multiple-Choice Procedure, which could limit the misuse of tiagabine alone or in combination with Δ^9 -THC. Further, repeated use of tiagabine for therapeutic purposes might be expected to produce a different response if cannabis was used concurrently, compared to acute dose combinations (Lile et al., 2011).

4.3. Performance impairment by tiagabine and tiagabine-cannabinoid combinations

When administered separately, tiagabine and Δ^9 -THC significantly impaired performance on the RA task and the DSST. These results are inconsistent with clinical trials indicating that tiagabine typically does not adversely affect cognition and motor control (Brunbech and Sabers, 2002). Important to note, however, is that research has focused on long-term treatment effects, rather than the impact of acute dosing, which also has important clinical implications (e.g., compliance; safety during initiation). Patients could have become tolerant to impairing effects of tiagabine that might have emerged when treatment was initiated. Moreover, most clinical trials with tiagabine have evaluated its effects in epileptic patients, who might be more likely to demonstrate improvements in cognition and motor control as a consequence of seizure management. Other side effects of tiagabine, alone and in combination with Δ^9 -THC, included significant increases in subject ratings of Sedated, as well as mild nausea that failed to reach statistical significance on the Visual Analog Scale but was reported by some subjects during the study debriefing. These results indicate that although tiagabine was generally safe and well tolerated when combined with Δ^9 -THC, side effects could emerge following concurrent administration of higher doses.

4.4. Limitations

Some limitations of the present study warrant mentioning. First, subjects were enrolled on an outpatient basis, so ongoing drug use could not be entirely controlled. Qualitative urine toxicology screening was conducted prior to the start of each experimental session to verify

that subjects had not used non-cannabis illicit drugs prior to the session, which limited the likelihood of interactions with experimentally administered drugs, but subjects could have used drugs having a short excretion half-lives during breaks between sessions (i.e., weekends) that might have gone undetected. In addition, the amount of cannabis used during participation likely varied across subjects. However, because subjects were frequent cannabis users and cannabis metabolites can be measured in urine for days to weeks after the cessation of use, it would have been impractical to require subjects to remain abstinent throughout the enrollment period. Second, a negative control condition (i.e., an active drug such as methylphenidate expected to engender Not Drug X responding; Lile et al., 2009; 2010) was not included to ensure that subjects were not simply reporting any active drug dose as Drug X. Important to emphasize, however, is that subjects were instructed to respond on the Not Drug X option in the absence of drug effects as well as when they experienced drug effects that differed from those of Drug X. These methods have been used successfully in previous studies to teach human subjects a selective discrimination based on the pharmacology of the training drug (e.g., Lile et al., 2009, 2010; Rush et al., 1998).

4.5. Therapeutic potential of GABA elevating compounds for cannabis-use disorders

As noted, tiagabine elevates synaptic GABA primarily via blockade of the GABA transporter. Other drugs that increase central GABA levels, albeit through different mechanisms, have been tested as potential medications for cannabis-use disorders. For example, valproic acid (as divalproex sodium, a 1:1 compound of valproic acid and its sodium salt), which inhibits the metabolic enzyme GABA transaminase (but see Rosenberg, 2007 for other potential mechanisms), has been evaluated in a human laboratory model of cannabis withdrawal and in a placebo-controlled clinical trial for cannabis dependence (Haney et al., 2004; Levin et al., 2004). Although divalproex sodium decreased self-reported craving for cannabis in the laboratory study, it also worsened mood and cognitive performance, and was ineffective at reducing cannabis use in the clinical trial. Gabapentin has also been examined in this regard. Although the principal mechanism of action of gabapentin is thought to be its ability to bind a particular subtype of voltage-gated calcium channels, there is also evidence that it increases the synthesis of GABA and stimulates GABA release (Taylor et al., 1998). In a study, published in abstract form (Mason, 2009), 12-week treatment with 1200 mg/day gabapentin was significantly more effective at reducing cannabis use and improving craving, depression and sleep quality than placebo. The degree to which the results from these studies with gabapentin can be attributed to GABA modulation, and therefore how they compare to studies with tiagabine, remains undetermined. However, the preliminary results demonstrating a reduction in cannabis use with gabapentin treatment are promising, particularly given a lack of medications to treat cannabis-use disorders.

4.6. Summary and Conclusions

Tiagabine enhanced the discriminative-stimulus, self-reported and performance effects of Δ^9 -THC, and produced overlapping effects when administered alone. To the extent that drugs that enhance the effects of Δ^9 -THC or produce comparable effects alone could address some of the complaints associated with cannabis abstinence thought to contribute to continued use (i.e., craving, irritability, anxiety, depression, difficulty sleeping), tiagabine should be considered for future clinical trials in cannabis-dependent patients seeking treatment. In addition, future studies with drugs selective for GABA receptors should be conducted to isolate the receptor subtype (i.e., GABA_A, GABA_B) responsible for the interaction with cannabinoids observed here. Such research will provide a better understanding of the neuropharmacology of cannabis and Δ^9 -THC in humans and help to identify more selective compounds for the management of cannabis-use disorders.

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Figure 1.

Separate and combined effects of Δ^9 -THC and tiagabine on Δ^9 -THC-appropriate responding on the drug-discrimination task. Filled symbols indicate values that are significantly different from placebo. Asterisks indicate combinations of Δ^9 -THC and tiagabine that are significantly different from that dose of Δ^9 -THC alone. The x-axis represents the Δ^9 -THC dose in mg; PL denotes placebo. Data points show means of 8 subjects. Uni-directional brackets indicate 1 SEM.

Figure 2.

Peak (maximum value) Visual Analog Scale ratings for Δ^9 -THC and tiagabine, alone and in combination, on the drug-effect questionnaire items Any Effect, High, Like Drug and Sedated. All other details are as in Figure 1.

Figure 3. Peak number of chains completed and total responses on the repeated acquisition task (minimum value) for Δ^9 -THC and tiagabine, alone and in combination. All other details are as in Figure 1.

Figure 4. Peak heart rate (maximum value) for Δ^9 -THC and tiagabine, alone and in combination. All other details are as in Figure 1.