Pharmacology Biochemistry and Behavior, Vol. 62, No. 3, pp. 473–480, 1999

0091-3057/99 $–see front matter

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PII S0091-3057(98)00195-6

Thujone Exhibits Low Affinity for Cannabinoid Receptors But Fails to Evoke Cannabimimetic Responses

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Received 10 April 1998; Revised 20 July 1998; Accepted 12 August 1998

MESCHLER, J. P. AND A. C. HOWLETT. *Thujone exhibits low affinity for cannabinoid receptors but fails to evoke cannabimimetic responses.* PHARMACOL BIOCHEM BEHAV **62**(3) 473–480, 1999.—Absinthe, an abused drug in the early 1900s, has been speculated to activate the receptors responsible for marijuana intoxication (the CB_1 cannabinoid receptor) (Nature 253:365–356; 1975). To test this hypothesis, we investigated oil of wormwood (*Artemisia absinthium*) the active plant product found in absinthe, and thujone, the active compound found in oil of wormwood. Radioligand receptor binding assays employing membrane preparations from rat brains containing CB_1 cannabinoid receptors, and human tonsils containing CB_2 receptors, demonstrated that thujone displaced [^{3}H]CP55940, a cannabinoid agonist, only at concentrations above 10 μM. HPLC analysis of oil of wormwood revealed that only the fractions having mobility close to thujone displaced [^{3}H]CP55940 from the CB_1 cannabinoid receptor. [^{35}S]GTPγS binding assays revealed that thujone failed to stimulate G-proteins even at 0.1 mM. Thujone failed to inhibit forskolin-stimulated adenylate cyclase activity in N18TG2 membranes at 1 mM. Rats administered thujone exhibited different behavioral characteristics compared with rats administered a potent cannabinoid agonist, levonantradol. Therefore, the hypothesis that activation of cannabinoid receptors is responsible for the intoxicating effects of thujone is not supported by the present data. © 1999 Elsevier Science Inc.

Cannabinoids Marijuana Absinthe Oil of wormwood Antinociception Levonantradol
G-proteins Adenylate cyclase CB_1 cannabinoid receptors Motor activity Thujone

ABSINTHE was a popular drug of abuse in the late 1800s and early 1900s, and was commonly used by poets and artists including Vincent Van Gogh (1). Although absinthe contained 80% ethanol, it was believed that ethanol-extractable components of herbal plants imparted a more complex profile of intoxicating effects than could be attributable to ethanol alone (2). Unlike ethanol intoxication, absinthe can cause hallucinations (29), and at higher doses, seizures (19,23). Analysis of absinthe revealed that oil of wormwood (the essential oils extracted from *Artemisia absinthium*) was the active plant product, and that thujone was the CNS-active component in the oil of wormwood (2). Because absinthe appeared to constitute an epidemic health problem, the distribution of absinthe was banned in many countries (29). Absinthe was later sold under the name of Pernod, but the herbal component of wormwood was not included (27), and in contrast to absinthe, the drink Pernod was not widely abused. However, in recent months, it appears that the illicit production and use of absinthe has been on the rise in European countries and the United States (10,26), and absinthe-related morbidity has been reported from use of oil of wormwood (30).

In 1975, an article by Del Castillo and co-workers (7) described a structural similarity between thujone and Δ^9-THC (Δ^9-tetrahydrocannabinol), the active component in marijuana (Fig. 1). These authors believed that the structural similarity between these two drugs might be substantial enough to hypothesize that both of these compounds worked through the same mechanism. Based on structural comparisons between the two drugs and anecdotal evidence suggesting that marijuana intoxication and absinthe intoxication were similar (i.e., both can cause hallucinations), they speculated that thujone and Δ^9-THC bound to the same receptor in the brain (7).

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Consistent with Del Castillo's observations, Rice and Wilson found that thujone has antinociceptive properties (23), and that (−)-thujone has 10-fold greater potency compared with its marginally active (+)-thujone isomer, providing further evidence that thujone acts through a specific receptor.

With the advent of the discovery of the CB1 cannabinoid receptor (8), and the elucidation of cannabinoid receptor-mediated signal transduction pathways (12–15), the hypothesis that thujone binds to cannabinoid receptors can now be easily tested. We define cannabinoid agonists by their ability to displace [^{3}H]CP55940 (a radiolabled cannabinoid ligand) from brain CB_1 cannabinoid receptors (14), their ability to activate G-proteins (25), and their ability to inhibit adenylate cyclase (18). Cannabinoid agonists also bind the CB_2 cannabinoid receptor found in immune cells and tissues (9) where they might modulate immune responses. Cannabinoid agonists can also be defined through behavioral testing in animals: cannabinoid agonists produce analgesia, hypomobility, and decreased locomotor activity in rats (6, 21).

METHOD

Materials

Synthetic (−)thujone was purchased from Sigma-Aldrich (St. Louis, MO), and oil of wormwood extract was acquired through Gaia Herbs, Inc. (Harvard, MA). Levonantradol was provided by Pfizer, and Tween 20 was purchased from Sigma. Male Sprague–Dawley rats (250–300 g) were purchased from Harlan (Indianapolis, IN).

Membrane Preparations

P2 membranes from rat brains were prepared using the method of Devane (8), and the protein concentrations were determined using the Bradford assay (4). Tonsil membranes were isolated from fresh human tonsils using the same method.

Receptor Binding Assays

[^{3}H]CP55940 ligand binding studies were performed using Regisil (Regis, Chicago, IL)-treated 96-well polypropylene plates. Each well contained (200 μl): TME buffer (20 mM TrisCl, pH 7.4, 3 mM $MgCl_2$, 1 mM Tris-EDTA) plus 0.1 mg/ml fatty acid-free bovine serum albumin (faf-BSA), 350 pM [^{3}H]CP55940 (New England Nuclear, Boston, MA), unlabeled ligands or vehicle as indicated, and 30 μg rat brain or human tonsil P2 membrane protein suspended in TME solution. [^{3}H]CP55940 was kept as a stock solution in ethanol, and before use was blown dry under N_2 gas and resuspended in 50 mg/ml faf-BSA and subsequently diluted 800-fold with TME buffer. [^{3}H]SR141716A binding assays were performed exactly as above, except the final concentration of [^{3}H]SR141716A was 2 nM. Thujone and oil of wormwood were diluted in 0.1 mg/ml faf-BSA in TME buffer. The 96-well plates were incubated at 30°C for 1 h with gentle shaking. After the incubation, 50 μl of 50 mg/ml BSA was added and the solution was washed onto a glass fiber "B" filter using a filtration harvester (Tomtec, Orange, CT). The filter was dried by microwave application for 3 min, and 50 μl of Wallac scintillation fluid was added to each filter square. The radioactivity on the filters was counted using a 1205 Betaplate (Wallac, Gaithersburg, MD). Each data point was determined in triplicate and each experiment was performed three times. Nonspecific binding was determined as the amount of radiolabled ligand that was not displaced by 0.1 μM desacetyllevonantradol (DALN), a potent cannabinoid agonist. Binding curves were analyzed using Inplot 4 (Graph Pad, San Diego, CA). K_i values were determined using an average of three experiments and employing the equation $K_i = IC_{50}/(1+([\text{radioligand}]/K_d \text{ of radioligand}))$. [^{3}H]CP55940 binds to the CB_1 receptor in rat brain membranes with a K_d = 350 pM and to the CB_2 receptor in human tonsil membranes with a K_d = 630 pM as determined by Scatchard analysis for these assay conditions. The K_d for SR141716A was 1 nM (11,24).

[^{35}S]GTPγS Binding Assay

Agonist-stimulated [^{35}S]GTPγS binding to G-proteins was performed employing a method adapted from Traynor (27,28). Each well of a 96-well 250-μl opaque polypropylene plate contained (100 μl): 0.375 nM [^{35}S]GTPγS, 1 mM dithiothreitol, 10 μM GDP, 10 μg rat brain P2 membrane protein, and the indicated concentrations of thujone or DALN, which had been diluted in 0.1 mg/ml faf-BSA in TME. The above mixture was incubated at 30°C on an orbital shaker for 1 h, then filtered through a glass fiber "B" filter using a Tomtec Harvester, and radioactivity was counted using a Wallac 1205 Betaplate. Curves were generated and EC_{50} values were determined using the Inplot 4 program.

Adenylate Cyclase Assay

Adenylate cyclase activity was measured, and percent inhibition was determined using the method of Howlett (17). Graphs were generated using the Inplot 4 software.

HPLC Fractionation

High-performance liquid chromatography was performed on a Varian (Palo Alto, CA) Star Workstation (9095 Auto-

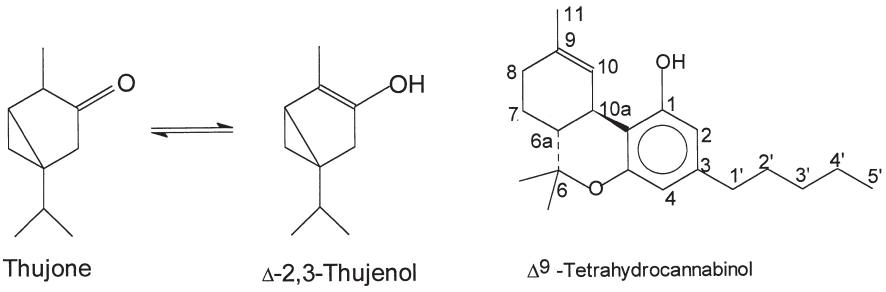

FIG. 1. Structures of thujone and the thujenol tautomer form (in equilibrium with thujone) and Δ^9-THC.

sampler, 9010 Solvent Delivery System, 9050 UV-VIS Detector). A Microsorb M-V C18 column was used, and absorbance was measured at 214 nm. Solvent A is 0.1% trifluoroacetic acid (TFA); solvent B is 80% acetonitrile, 0.085% TFA (filtered and degassed prior to use). The elution gradient profile was as follows (1 ml/min): 0 to 5 min, 100% solvent A; 5 to 30 min, 100% solvent A to 100% solvent B gradient; 30 to 35 min, 100% solvent B. The oils thujone or oil of wormwood were diluted 1:10 (vol/vol) in acetonitrile followed by a 1:100 dilution in solvent A; a 100-μl volume was injected onto the column. One-minute fractions were collected, lyophilized, resuspended in 0.1 ml 0.1 mg/ml faf-BSA in TME, and assayed for binding according to the method described above. A synthetic thujone standard was run to determine the retention time of thujone.

Behaviors in Rats

Male Sprague–Dawley rats were treated with thujone, levonantradol, or vehicle (5% ethanol, 5% Tween-20, and 90% sterile saline). The solutions were administered intraperitoneally in coded syringes so that data would be collected in a blinded fashion. The rats were tested in three different behavioral paradigms sequentially, beginning 30 min after drug administration. To determine the fraction of time the rats remained immobile, the ring stand test was performed according to Pertwee (22), except that the height of the ring stand was adjusted for rats (48 cm and the diameter of the ring was 12.5 cm). To determine locomotor activity, the rats were placed in a cylindrical open field (inner diameter 33.5 cm and outer diameter 59.5 cm) with four evenly spaced lines. The number of lines crossed was recorded for a 5-min testing period. To determine analgesia, the hot-plate test (20) was performed at a temperature of 52°C, and the number of seconds before the rat engaged in paw licking was recorded as the paw-lick latency. To avoid unnecessary paw injury, the maximum testing period was 30 s, at which time the rat was removed and paw-lick latency was recorded as 30. Results from all behavioral tests were graphed using Prism (GraphPad) and analyzed by one-way ANOVA followed by the Dunnett's post hoc test using INSTAT 2 (GraphPad) to determine statistical significance.

RESULTS

Affinity of Thujone for Cannabinoid Receptors

We first investigated the affinity of thujone for the brain CB_1 cannabinoid receptor by displacement of the radiolabled cannabinoid ligand [^{3}H]CP55940. Concentrations of thujone less than 10 μM failed to displace the radiolabled ligand (Fig. 2A). Thujone, at 1 mM, effectively displaced the ligand, and the K_i was 130 μM, with a Hill coefficient of −1. We also wanted to determine if thujone could displace an antagonist radioligand at the CB_1 cannabinoid receptor using [^{3}H]SR141716A. Thujone displaced [^{3}H]SR141716A at concentrations between 10 μM and 1 mM, with a K_i of 165 μM and a Hill coefficient of −1 (Fig. 2B). Because two subtypes of cannabinoid receptors exist, we wanted to determine whether thujone could bind to the CB_2 cannabinoid receptor. To test this hypothesis, we determined thujone's ability to displace [^{3}H]CP55940 using human tonsil membrane preparations that possess CB_2 but not CB_1 cannabinoid receptors. Thujone displaced [^{3}H]CP55940 at the CB_2 receptor at greater than 10 μM concentrations, and exhibited a K_i of 115 μM with a Hill coefficient of −1 (Fig. 3). The affinity of thujone for the CB_2 cannabinoid receptor is approximately the same as for the CB_1 cannabinoid receptor.

Because it is possible that thujone is not the only active component of oil of wormwood, we determined whether any constituents of oil of wormwood could displace [^{3}H]CP55940 from the CB_1 cannabinoid receptor (Fig. 4). At 1:1000 or greater dilutions, there was little displacement of [^{3}H]CP55940; however, at a 1:100 dilution there was a dramatic displacement of [^{3}H]CP55940. The Hill coefficient for this displacement curve was between −2 and −3, which is atypical for a single-site ligand–receptor interaction. It was noted that at a 1:10 dilution, nonspecific binding of [^{3}H]CP55940 appeared to be displaced, suggesting some interference with the assay procedure.

In an effort to determine what components in the oil of wormwood can displace [^{3}H]CP55940 at the CB_1 cannabinoid receptor, HPLC analysis was performed. As a control, HPLC analysis was performed using synthetic thujone, which demonstrated that the retention time of thujone in this solvent system was 24.5 min. According to the quantitative analytical specifications, the contaminants at 26.5 and 27.1 min are

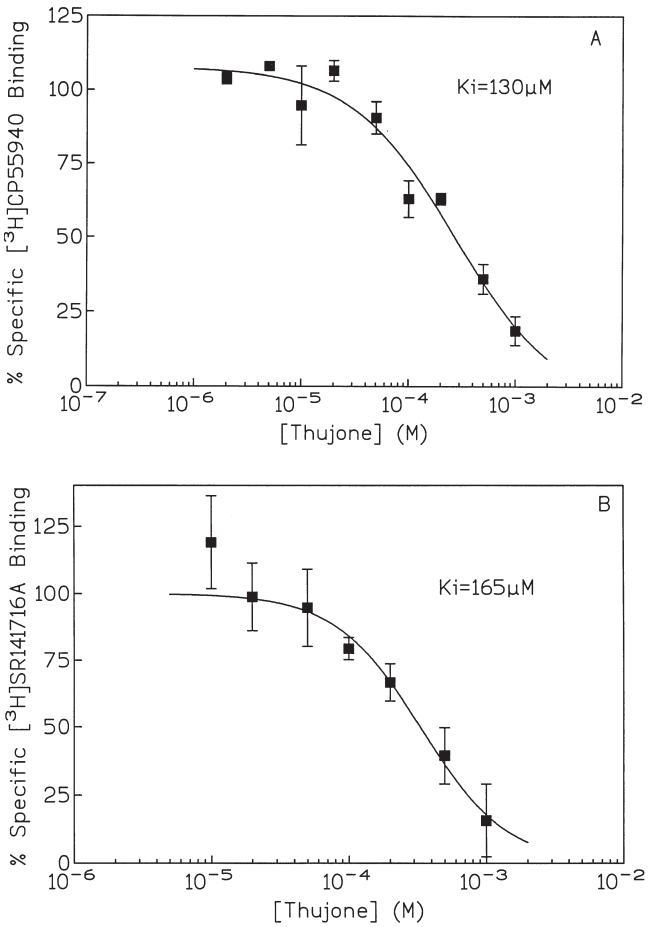

FIG. 2. Ability of thujone to bind to the CB_1 cannabinoid receptor. (A) Displacement of the cannabinoid receptor agonist [^{3}H]CP55940 by thujone. (B) Displacement of the CB_1 cannabinoid receptor antagonist [^{3}H]SR141716A by thujone. Data points represent the mean ± SEM of three independent experiments in which each drug concentration was performed in quadruplicate.

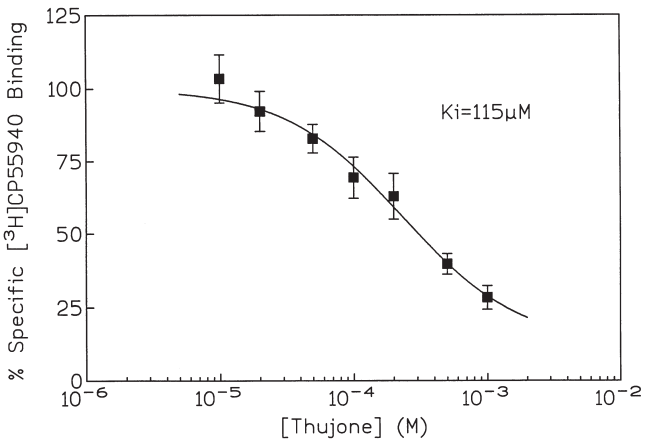

FIG. 3. Displacement by thujone of [^{3}H]CP55940 from CB_2 cannabinoid receptors in human tonsil membrane preparations. These data represent the mean ± SEM of three independent experiments in which each drug concentration was performed in quadruplicate.

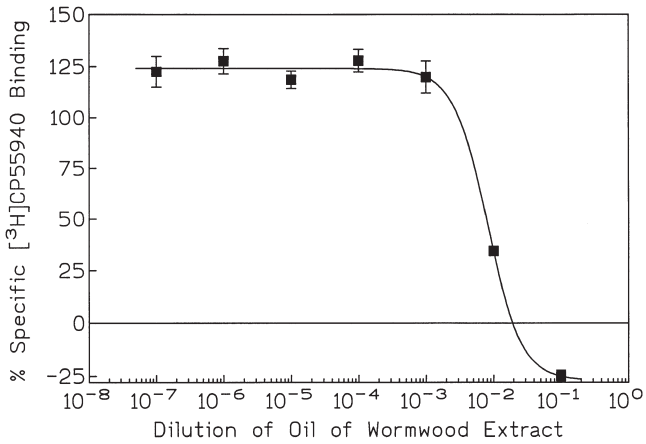

FIG. 4. Effect of dilutions of oil of wormwood on CB_1 cannabinoid receptor binding of [^{3}H]CP55940. These data represent the mean ± SEM of quadruplicate determinations from a single representative experiment.

bornyl acetate and fenchone, and the minor components are camphor, p-cymene, and limonene, all of which are byproducts of thujone synthesis (Fig. 5A). Oil of wormwood was fractionated under identical conditions (Fig. 5B), and 1-min fractions were collected. [^{3}H]CP55940 displacement binding assays were conducted on aliquots of each fraction that would be equivalent to a 1:100 dilution based upon original volume (Fig. 5C). A fraction of oil of wormwood that displaced [^{3}H]CP55940 by about 45% had a similar retention time to the synthetic thujone standard (24.5 min). The oil of wormwood peak at 24.5 min, believed to correspond to thujone, was run once again on the HPLC column, and it exhibited the same retention time as it did previously (data not shown). Displacement also occurred with fractions that correspond to peaks appearing at earlier retention times; these peaks may comprise related terpenoid components of the oil. A set of peaks appearing early in the elution failed to displace [^{3}H]CP55940. Because neither the peak corresponding to thujone nor any other peak displaced more than 50%, no effort was made to further purify these fractions. From these data we conclude that thujone and other relatively nonpolar components of the oil of wormwood displace [^{3}H]CP55940 at concentrations that would be consistent with a low-affinity interaction with the CB_1 cannabinoid receptor.

One caveat to the determination of meaningful affinity constants from these radioligand binding studies with such low-affinity ligands is that thujone is practically insoluble in water (5). During serial dilution, high concentrations of thujone (0.1 mM) or low dilutions of the oil of wormwood extract (1:10) appeared cloudy, suggesting that these compounds existed as a suspension with faf-BSA in aqueous media at these high concentrations. Thujone, being lipophilic, most likely accumulates in the lipid phase of the bilayer when incubated with membranes in an aqueous media, suggesting that the calculated concentration in the media and the actual concentration will be different. Caution must be exercised before drawing conclusions regarding the mechanism by which thujone alters binding of [^{3}H]CP55940 and [^{3}H]SR141716A to the CB_1 receptor at these high concentrations. The HPLC fractions from oil of wormwood that displaced [^{3}H]CP55940 exhibited similar retention times as thujone, indicative of their nonpolar properties. The components of oil of wormwood displaced [^{3}H]CP55940 with a Hill coefficient of −2, suggesting that a simple interaction with the radioligand binding site by the hydrophobic compounds may not fully explain the results. Rather, other factors may be influencing the amount of [^{3}H]CP55940 bound to the receptor, such as perturbation of membrane fluidity, displacement of annular lipids surrounding the receptor protein, or sequestration of the hydrophobic radioligand by the constituents in oil of wormwood.

Signal Transduction by Thujone

If thujone is able to behave as an agonist to produce biological responses via the CB_1 cannabinoid receptor, it would be able to activate G-proteins in rat brain membranes as does the cannabinoid agonist, DALN. We tested this employing the [^{35}S]GTPγS binding assay at various concentrations of thujone. This assay is based upon the principle that the agonist–receptor complex evokes the dissociation of GDP from the G-protein heterotrimer, thereby allowing GTP or its analogs to occupy that site. GTPγS fails to be hydrolyzed to GDP by the α subunit of G-proteins, and thus, allows accumulation of [^{35}S]GTPγS-labeled G-proteins as a measure of receptor-mediated activity. Figure 6 demonstrates that although DALN increased [^{35}S]GTPγS binding, thujone was unable to stimulate [^{35}S]GTPγS binding at concentrations as high as 100 μM.

Because CB_1 receptor agonists inhibit adenylate cyclase (and consequently cAMP formation) via the G-protein G_i, it was important to determine if thujone could also inhibit adenylate cyclase. Figure 7 demonstrates that DALN inhibited adenylate cyclase in N18TG2 membranes maximally, with 38% inhibition at 1 μM (17). Thujone failed to significantly inhibit adenylate cyclase activity at concentrations as high as 1 mM.

Additionally, thujone was tested as a potential antagonist for cannabinoid agonist-mediated responses. Thujone failed to antagonize cannabinoid agonist (DALN)-stimulated [^{35}S]GTPγS binding at doses as high as 100 μM (data not shown).

Behavioral Effects of Thujone

To determine if thujone exhibits any cannabimimetic activity in vivo, thujone was compared with levonantradol, a po-

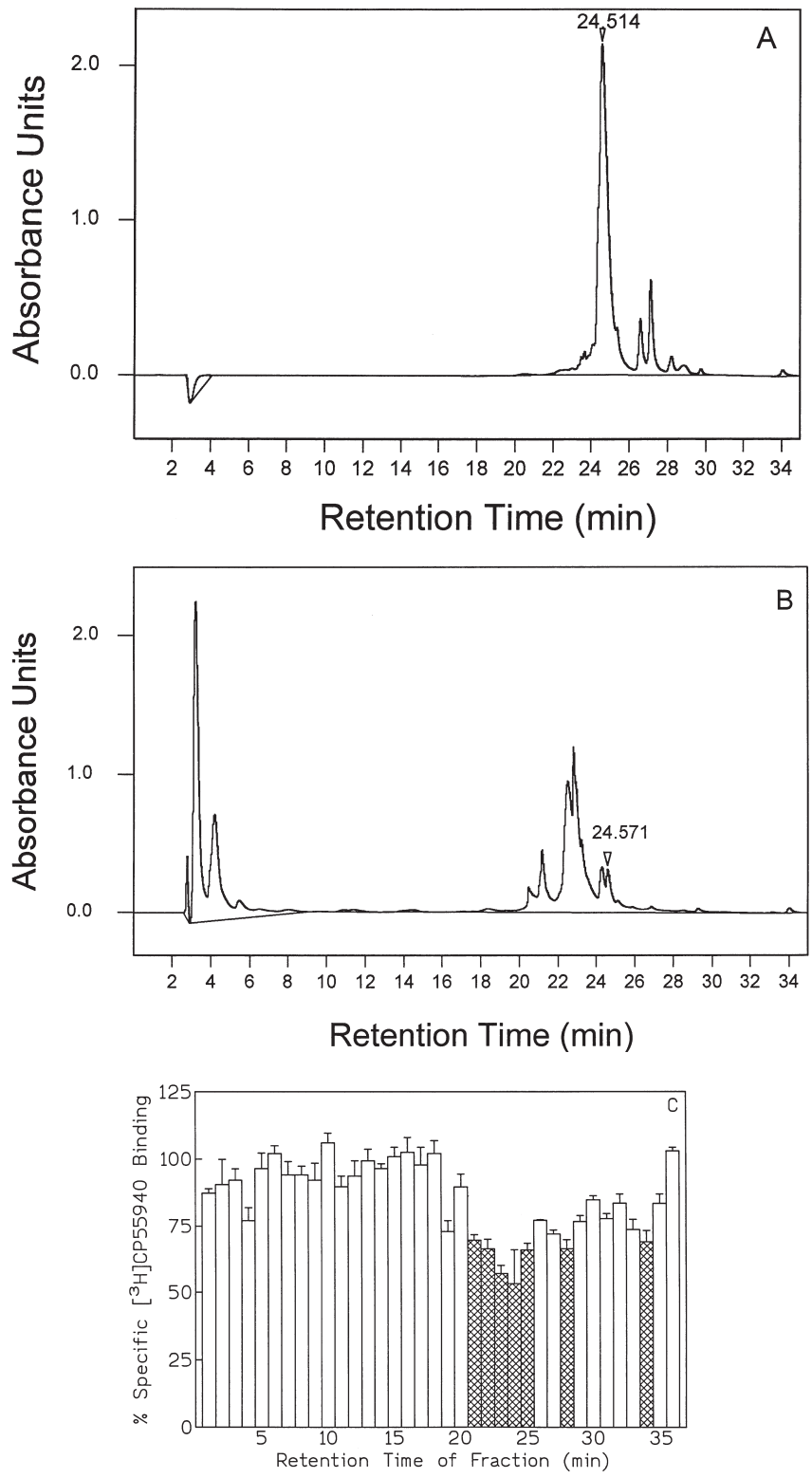

FIG. 5. HPLC analysis of thujone and oil of wormwood. (A) HPLC analysis of synthetic thujone. This HPLC trace is representative of two separate injections of thujone standard. (B) HPLC fractionation of oil of wormwood. The HPLC trace is a representation of two independent HPLC analyses of oil of wormwood. C. Determination of binding to the CB_1 cannabinoid receptor of the HPLC fractions from (B). All fractions displacing more than 30% of [^{3}H]CP55940 specific binding are hatched. The binding data are the mean ± SEM of quadruplicate determinations from one representative radioligand binding experiment.

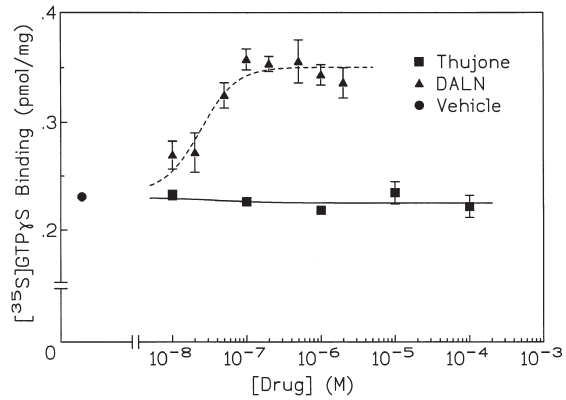

FIG. 6. Effects of the cannabinoid agonist DALN and of thujone on [^{35}S]GTP S binding to G-proteins. These data points are the mean ± SEM of quadruplicate determinations from one of three independent experiments.

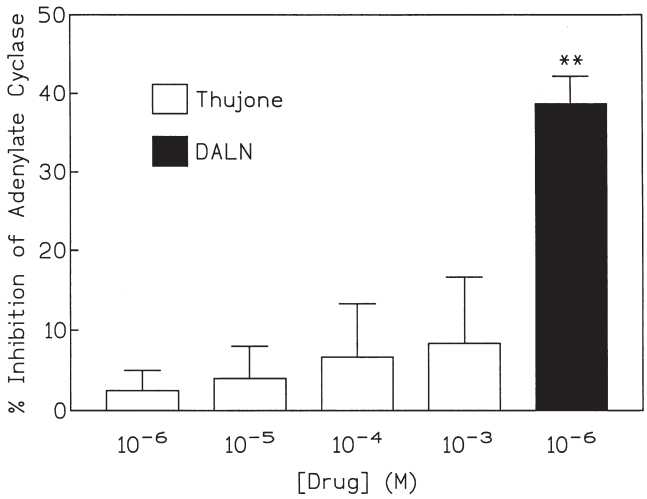

FIG. 7. Effects of DALN and thujone on forskolin-stimulated adenylate cyclase activity. Data are presented as the % inhibition of forskolin (10 μM)-stimulated adenylate cyclase activity determined using N18TG2 neuroblastoma membranes. Data are the mean ± SEM of results from three experiments, each performed with triplicate determinations, and statistical significance was determined by Dunnett's post hoc test. DALN produced a significant inhibition of adenylate cyclase activity denoted by a **$F(5, 16) = 8.44$, $p < 0.01$; thujone failed to produce significant inhibition of adenylate cyclase activity at doses as high as 1 mM.

tent cannabinoid agonist (16), in a battery of tests used to describe CB_1 receptor-mediated responses in rodents (6,21). Three tests were used to assess cannabinoid agonist activity in male Sprague–Dawley rats: the open-field test for locomotor activity, the ring-stand test for immobility (catalepsy), and hot-plate test for antinociception. Although the cannabinoid agonist levonantradol produced significant decreases in locomotor activity as measured by the open-field test, thujone produced no significant changes from vehicle (Table 1). In the ring-stand test, levonantradol produced significant increases in the fraction of time spent immobile on the ring stand compared with vehicle (Table 1). Thujone did not cause a significant increase in the immobility index. Levonantradol produced significant analgesia as measured in the hot-plate test; however, the response to thujone was no different from vehicle (Table 1). Higher doses of thujone were not tested because at the highest doses of 10 and 30 mg/kg, thujone was difficult to dissolve in the vehicle, and the drug had to be delivered as a "cloudy" suspension. Additionally, one rat of five given a dose of 30 mg/kg thujone convulsed, and therefore, we terminated the dose–response protocols at this dose.

DISCUSSION

The physiological relevance of cannabinoid receptors in the etiology of acute absinthe intoxication can be considered based upon our present findings. The present data demon-

TABLE 1

EFFECTS OF LEVONANTRADOL AND THUJONE ON MOTOR ACTIVITY, IMMOBILITY, AND ANALGESIA IN RATS DETERMINED USING THE OPEN-FIELD, RING-STAND, AND HOT-PLATE TESTS, RESPECTIVELY

	Open-Field Test mean ± SEM	Ring Stand Test mean ± SEM	Hot-Plate Test mean ± SEM
Vehicle	47.4 ± 19.6	0.0011 ± 0.0011	14.0 ± 9.3
Levonantradol (0.3 mg/kg)	7 ± 6.9*	0.4800 ± 0.1304†	28.9 ± 3.9‡
Thujone (0.1 mg/kg)	49.0 ± 11.5	ND	13.6 ± 9.6
Thujone (0.3 mg/kg)	38.0 ± 13.0	ND	17.7 ± 10.2
Thujone (1.0 mg/kg)	47.2 ±12.8	ND	14.7 ±6.8
Thujone (3.0 mg/kg)	37.8 ± 23.7	0 ± 0	13.7 ± 9.0
Thujone (10 mg/kg)	36.8 ± 22.5	0 ± 0	16.0 ± 9.9
Thujone (30 mg/kg)	ND	ND	16.4 ± 9.6

Levonantradol (0.3 mg/kg) *exhibited a significant decrease in the number of line crossings compared to vehicle in the open field, $F(6, 23) = 2.63$, $p < 0.05$;) †exhibited a significant increase in the immobility index compared to vehicle in the ring stand test, $F(3, 13) = 51.76$, $p < 0.01$; and ‡produced a significant increase in paw-lick latency compared to vehicle in the hot plate test, $F(7, 65) = 4.41$, $p < 0.05$. No significant differences ($p > 0.05$) were found between vehicle compared with any of the doses of thujone in any of the three behavioral tests.

ND = not determined.

strate that thujone, the active component of absinthe, exhibits a low affinity for cannabinoid receptors, displacing [^{3}H]CP55940 at CB_1 and CB_2 receptors and [^{3}H]SR141716A from CB_1 receptors at concentrations of 10 μM or greater. Nevertheless, thujone fails to exhibit a significant effect on G-protein or adenylate cyclase activity at concentrations as great as 1 mM, and fails to elicit typical cannabinoid receptor-mediated responses in rodents at doses as high as 30 mg/kg. One can estimate whether such concentrations might be achieved in the tissues of absinthe drinkers. A 70-kg human could consume a maximum of 200 ml of alcoholic absinthe in one episode, a volume limited by the 80% ethanol content. A concentration of thujone of 2.4 mM (360 mg/l) in alcohol solution has been estimated for typical absinthe preparations (1,3). The maximum attainable intake would be equivalent to a dose of 1 mg/kg in a human subject. If thujone were to distribute in a volume of distribution equal to the total body water without metabolism, sequestration or excretion, then the maximum blood concentration of thujone in a single imbibition would be approximately 10 μM. However, due to its lipid solubility, it is likely that a significant fraction of thujone becomes sequestered in body fat during circulation. Other investigators have suggested the possibility for hepatic first-pass extraction, further limiting the quantity of thujone available for distribution to the brain (3). Hence, it is not likely that a direct, low-affinity interaction with cannabinoid receptors in the brain can be considered as the primary mechanism of action of thujone and related compounds in absinthe intoxication.

The behavioral studies reported here using a rodent model demonstrate that the effects of thujone are different from those of a cannabinoid agonist, levonantradol. Levonantradol and related cannabinoid agonists produce immobility, analgesia, and decreased locomotor activity, whereas thujone-treated rats did not display this triad of behavioral changes. However, our analgesia studies are inconsistent with the results obtained by Rice and Wilson (23), who demonstrated analgesia from thujone with an ED_{50} of 6.5 mg/kg in mice. Possible explanations for this discrepancy are that our data were obtained using Sprague–Dawley rats, whereas Rice and Wilson tested mice, and our hot-plate test was administered at a setting of 52°C, whereas Rice and Wilson used a setting of 55°C. These differences might suggest a possible species or strain sensitivity to thujone in this particular response.

Thujone has been reported as a putative stimulant of the central nervous system based on its convulsant properties (19). In the present studies, the open-field test did not demonstrate increases in locomotor activity elicited by thujone. One rat experienced a tonic–clonic seizure under the influence of 30 mg/kg thujone. There are a number of potential mechanisms of action for any convulsant drug. Convulsants may antagonize the GABAergic or glycinergic systems that provide a tonic inhibitory influence on synaptic activity; or convulsants may activate the glutamatergic, the dopaminergic or the adrenergic systems. Another possibility is that by intercalating in membranes, thujone may perturb neuronal membrane fluidity, leading to a release of excitatory neurotransmitters and possible seizure activity. However, from previous studies demonstrating that the (−) isomer has a greater ability to elicit seizures than the (+) isomer (23), it is more likely that the convulsive effect of thujone involves a specific receptor system.

We can conclude that the hypothesis that thujone activates the same receptor system as Δ^9-THC is incorrect, based on the findings that neither thujone nor oil of wormwood bind to and activate brain cannabinoid receptors at physiologically relevant concentrations, and that thujone fails to elicit cannabimimetic behavioral effects in animals at physiologically relevant doses. Thus, the neuropharmacological mechanism of action of thujone has yet to be determined. Imbibers of absinthe claimed that there were subjective effects of absinthe intoxication other than those attributable to the effects of alcohol. This is witnessed by the drop in absinthe popularity when wormwood (and hence thujone) was removed from the formula and it was sold as Pernod (29). However, in recent months, the use of absinthe seems to be returning (10,26). Oil of wormwood can be purchased from health food stores as an aroma therapy or homeopathic remedy that can be taken orally or absorbed through the skin (30). Due to the ease of availability of oil of wormwood, increasing incidence of morbidity may occur.

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