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Effects of SR141716A on Diazepam Substitution for Δ^9-Tetrahydrocannabinol in Rat Drug Discrimination

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WILEY, J. L. AND B. R. MARTIN. *Effects of SR141716A on diazepam substitution for Δ⁹-tetrahydrocannabinol in rat drug discrimination*. PHARMACOL BIOCHEM BEHAV **64**(3) 519–522, 1999.—Interaction of cannabinoids with GABAergic systems has been noted in a number of previous studies. In the present study, this interaction was examined in a drug-discrimination paradigm. Rats were trained to discriminate either Δ^9-tetrahydrocannabinol (Δ^9-THC; 3 mg/kg) or diazepam (2.5 mg/kg) from vehicle in two-lever drug discrimination procedures for food reinforcement. As in previous studies, diazepam partially substituted for Δ^9-THC, but only at high doses that also decreased response rates. In contrast, Δ^9-THC did not substitute for diazepam in any of the rats. Hence, cross-generalization of these two drugs was asymmetrical. When tested in combination with diazepam, the brain cannabinoid (CB1) receptor antagonist SR141716A did not block the partial substitution of diazepam for Δ^9-THC, nor did it antagonize the discriminative stimulus effects of diazepam in diazepam-trained rats. These results suggest that the partial overlap in the discriminative stimulus effects of Δ^9-THC and diazepam is not mediated by diazepam action at CB1 receptors. However, the fact that diazepam produced partial substitution for Δ^9-THC is consistent with a GABAergic component to cannabinoid drug discrimination. © 1999 Elsevier Science Inc.

Cannabinoid Tetrahydrocannabinol Diazepam GABA SR141716A

DISCRIMINATION with Δ^9-tetrahydrocannabinol (Δ^9-THC), the primary psychoactive substituent of *Cannabis sativa*, represents an animal model of marijuana intoxication in humans (1). Potency of cannabinoid compounds to substitute for Δ^9-THC is highly correlated with their potency for producing subjective marijuana-like effects in humans (6). Further, Δ^9-THC discrimination is pharmacologically selective for psychoactive cannabinoids, as most noncannabinoid compounds do not substitute for Δ^9-THC in rats trained to discriminate this cannabinoid drug from vehicle (3,4). One notable exception is the benzodiazepine, diazepam. Diazepam produces consistent, but partial, substitution for cannabinoids in rats trained to discriminate Δ^9-THC (3,10) or the synthetic cannabinoid CP 55,940 (19) from vehicle. The effect can be attenuated by flumazenil, a benzodiazepine antagonist, and is believed to be related to interaction of the cannabinoid and GABAergic systems (10). The purpose of the present study was two-fold: 1) to determine whether Δ^9-THC would partially substitute for diazepam in rats trained to discriminate diazepam from vehicle (i.e., partial crossgeneralization), and 2) to evaluate the ability of a cannabinoid antagonist, SR141716A, to block any substitution or partial substitution of diazepam that was observed in rats trained to discriminate Δ^9-THC from vehicle or diazepam from vehicle. A preliminary report of these results was published in abstract form (18).

METHOD

Subjects

Adult male Sprague–Dawley and Long–Evans rats (290–350 g), obtained from Harlan (Dublin, VA), were individually housed in a temperature-controlled (20–22°C) environment with a 12-h light–dark cycle (lights on at 0700 h). Rats were maintained within the indicated weight range by restricted postsession feeding. Rats were drug naive at the beginning of the study.

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Apparatus

Standard operant conditioning chambers (Lafayette Instruments Co., Lafayette, IN) were housed in sound-attenuated cubicles. A pellet dispenser delivered 45-mg BIO SERV (Frenchtown, NJ) food pellets to a food cup located between two response levers mounted on the front wall of the chamber. Fan motors provided ventilation and masking noise for each chamber. Four-watt house lights were located above each lever and were illuminated during training and testing sessions. A microcomputer with Logic "1" interface (MED Associates, Georgia, VT) and MED-PC software (MED Associates) were used to control schedule contingencies and to record data.

Drugs

Δ^9-THC (National Institute on Drug Abuse, Rockville, MD) and SR141716A (Pfizer Inc., Groton, CT) were dissolved in a 1:1:18 vehicle mixture of absolute ethanol, Emulphor-620 (Rhone-Poulene, Inc., Princeton, NJ), and saline. This 1:1:18 mixture also was used as vehicle during training. A stock solution of diazepam, 5 mg/ml (Schein Pharmaceutical Inc., Port Washington, NY) was purchased commercially. Lower doses were obtained by dilution with saline. Higher doses were obtained by adjustment of the volume of injection of the stock solution. All drugs were administered intraperitoneally (IP) at a volume of 1 ml/kg unless otherwise noted.

Procedure

Three groups of adult male rats were food-restricted and were trained to discriminate Δ^9-THC (3 mg/kg, IP, two groups) from vehicle or diazepam (2.5 mg/kg, IP, 1 group) from vehicle in two-lever drug discrimination procedures. They were trained and tested during 15-min sessions under an FR-10 schedule of food reinforcement [see (21) for additional procedural details]. Following successful acquisition of the discrimination, stimulus substitution tests were conducted twice weekly, typically on Tuesdays and Fridays. Training continued on intervening weekdays. During test sessions, 10 consecutive responses on either lever delivered reinforcement. Doses of each test drug were usually administered in ascending order. Control tests with the training dose of Δ^9-THC or diazepam and vehicle were conducted before each dose–effect curve determination. In the first group of Δ^9-THC-trained Sprague–Dawley rats (n = 8), substitution dose–effect curve determinations were performed with Δ^9-THC and diazepam. Then, combinations of diazepam doses and 1 mg/kg SR141716A were tested. In the second group of Δ^9-THC-trained Long–Evans rats (n = 4), combinations of diazepam doses and 10 mg/kg SR141716A were tested. In the diazepam-trained Sprague–Dawley rats (n = 8), substitution dose–effect curve determinations were performed with diazepam and Δ^9-THC. Then, combinations of the training dose of diazepam (2.5 mg/kg) and different doses of SR141716A were tested. In all discrimination groups, diazepam and Δ^9-THC were administered IP 30 min prior to the start of the test session and SR141716A was administered IP 40 min pre-session.

Data Analysis

For each test session, mean percentage of responses on the drug lever and response rate (responses/s) were calculated. (Data on lever selection for rats that had ≤0.02 responses/s during a test session were excluded from group averages for lever selection.)

RESULTS

Figure 1 shows the results of substitution tests with Δ^9-THC (■), diazepam (□), diazepam + 1 mg/kg SR141716A (●), and diazepam + 10 mg/kg SR141716A (○) on percentage of Δ^9-THC lever responding (top panel) and response rates (bottom panel) in rats trained to discriminate Δ^9-THC from vehicle. As expected, Δ^9-THC produced dose-dependent full substitution for the training dose (3 mg/kg), with decreases in response rates occurring at higher doses. In contrast, diazepam produced only partial substitution for Δ^9-THC (63–66% drug-lever responding; Fig. 1, top panel) at higher doses (3, 5.6, and 10 mg/kg). The 5.6 and 10 mg/kg doses of diazepam severely decreased overall rates of responding in most rats (Fig. 1, bottom panel). SR141716A (1 mg/kg) failed to block partial substitution by the 5.6 mg/kg dose of diazepam, and only slightly decreased the degree of substitution produced by the 3 mg/kg dose. Similarly, SR141716A (10 mg/kg) also did not block partial substitution of 3 or 5.6 mg/kg diazepam in a second group

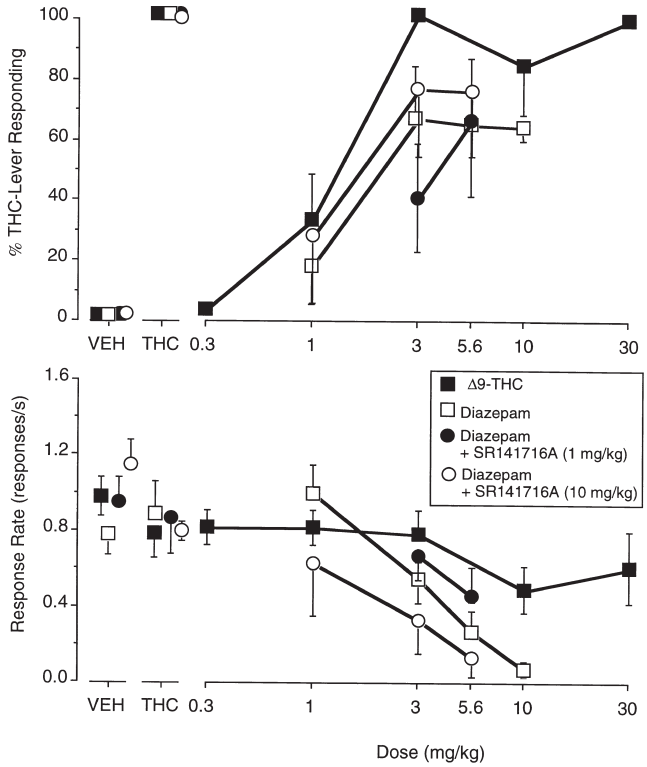

FIG. 1. Results of substitution tests with Δ^9-THC (■), diazepam alone (□), and diazepam + 1 mg/kg SR141716A (●) on percentage of Δ^9-THC-lever responding (top panel) and response rates (bottom panel) in rats trained to discriminate Δ^9-THC from vehicle. Combinations of diazepam + 10 mg/kg SR141716A (○) were tested in a second group of rats trained to discriminate Δ^9-THC from vehicle under similar experimental conditions. Points above VEH and THC represent the results of control tests with vehicle and 3 mg/kg Δ^9-THC conducted before each dose–effect curve determination. Each value in the Δ^9-THC, diazepam alone, and diazepam + 1 mg/kg SR141716A combination curves represents the mean (±SEM) of data from six to eight rats, except for percentage of THC lever responding for 5.6 mg/kg diazepam (n = 4) and 10 mg/kg diazepam (n = 2). Each value in the diazepam + 10 mg/kg SR141716A combination curve represents the mean (±SEM) of data from four rats, except for percentage of THC lever responding for 3 mg/kg diazepam combination (n = 2) and 5.6 mg/kg diazepam combination (n = 1).

of rats. Stimulus control was maintained throughout both Δ^9-THC discrimination experiments, as rats responded predominantly on the injection-appropriate lever during control tests with vehicle and 3 mg/kg Δ^9-THC.

Figure 2 shows the results of substitution tests with diazepam (□), Δ^9-THC (■), and diazepam + SR141716A (●) on percentage of diazepam-lever responding (top panel) and response rates (bottom panel) in rats trained to discriminate diazepam (2.5 mg/kg) from vehicle. Diazepam fully substituted for itself (top panel) and decreased overall rates of responding at higher doses (bottom panel). The training dose (2.5 mg/kg) of diazepam produced responding almost exclusively on the drug lever. SR141716A (1–10 mg/kg) did not attenuate this high degree of substitution. Δ^9-THC failed to substitute (fully or partially) for diazepam, even at response rate decreasing doses. Stimulus controls was maintained throughout the diazepam discrimination experiment, as rats responded predominantly on the injection-appropriate lever during control tests with vehicle and 2.5 mg/kg diazepam.

DISCUSSION

The partial substitution of diazepam for Δ^9-THC is consistent with a number of previous discrimination studies in which diazepam has been shown to mimic the discriminative stimulus effects of Δ^9-THC in some (but not all) animals tested in this procedure. This effect occurs reliably in rats and in rhesus monkeys (3,10,20), but not in pigeons (8). The exact mechanism for the partial overlap of the discriminative stimulus effects of benzodiazepines with those of cannabinoids is not entirely clear, but several lines of evidence suggest that, in addition to their well-characterized effects on brain cannabinoid (CB1) receptors, cannabinoids interact with GABAergic systems. First, Revuelta et al. (16) have shown that Δ^9-THC increases GABA turnover in rat brain, suggesting that cannabinoids may enhance GABA release or inhibit its reuptake (2). Second, potentiation of the cataleptic and hypothermic effects of Δ^9-THC in mice has been observed following administration of benzodiazepines (11,13,15). Further, flumazenil antagonizes the effects of diazepam in these mouse procedures (15), as well as its partial substitution for Δ^9-THC in cannabinoid discrimination procedures (10). In contrast, flumazenil does not affect the substitution of Δ^9-THC for itself in Δ^9-THC–trained rats (10), nor does it antagonize the subjective high produced by marijuana in humans (7). Hence, the partial substitution of diazepam for Δ^9-THC appears to involve action of diazepam at the benzodiazepine site of the GABA receptor complex rather than diazepam interaction with cannabinoid receptors.

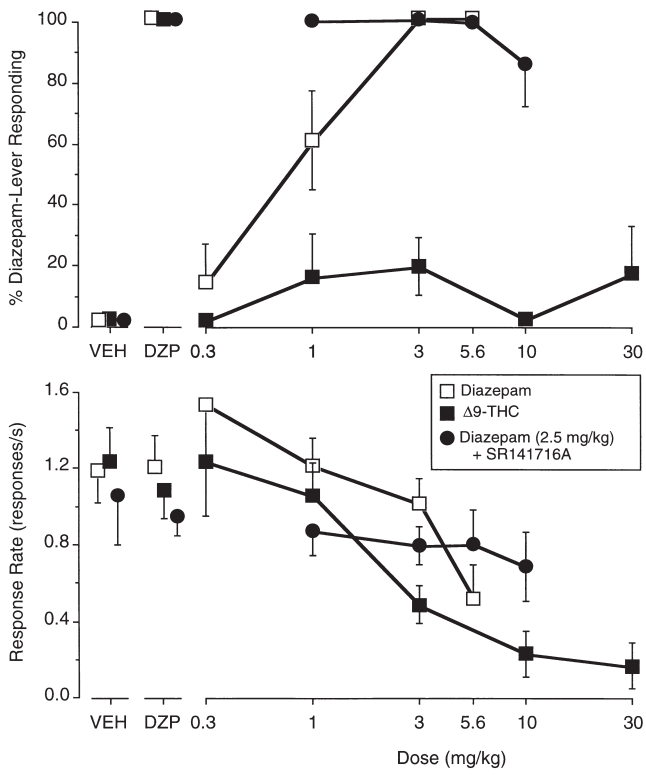

FIG. 2. Results of substitution tests with diazepam alone (□), Δ^9-THC alone (■), and 2.5 mg/kg dose of diazepam + SR141716A (●) on percentage of diazepam lever responding (top panel) and response rates (bottom panel) in rats trained to discriminate diazepam (2.5 mg/kg) from vehicle. Points above VEH and DZP represent the results of control tests with vehicle and 2.5 mg/kg diazepam conducted before each dose–effect curve determination. Each value represents the mean (±SEM) of data from seven to eight rats, except for percentage of THC lever responding for 5.6 mg/kg diazepam (n = 5), 10 mg/kg Δ^9-THC (n = 3), and 30 mg/kg Δ^9-THC (n = 3).

Indeed, diazepam and other benzodiazepines do not have any direct effects on cannabinoid CB1 receptors. Whereas previous studies have shown that the cannabinoid CB1 receptor antagonist SR141716A completely and dose dependently blocked the discriminative stimulus effects of Δ^9-THC (9,12,21), in the present study it did not attenuate the substitution of diazepam for Δ^9-THC. SR141716A also did not substantially affect the response rate decreases that accompanied this substitution. These results suggest that the cannabimimetic effects of Δ^9-THC and diazepam are differentially mediated by cannabinoid CB1 receptors and benzodiazepine receptor sites, respectively.

In contrast to the reliable partial substitution of diazepam for Δ^9-THC in rats trained to discriminate Δ^9-THC from vehicle, Δ^9-THC did not substitute, even partially, for diazepam in rats trained to discriminate diazepam from vehicle. In addition, doses of SR141716A up to 10 mg/kg (IP) had no effect on the diazepam-like discriminative stimulus effects of the training dose of diazepam. In previous studies, SR141716A also did not block the pharmacological effects of other noncannabinoid drugs, including haloperidol, reserpine, oxotremorine, and apomorphine (5,17), suggesting that this drug selectively attenuates cannabinoid-induced behaviors. In combination with the results of the Δ^9-THC discrimination study, these diazepam discrimination results suggest that GABAergic interaction may facilitate, but is not crucial for, cannabimimetic discriminative stimulus effects.

In summary, results of in vivo studies offer support for an interaction between cannabinoid and GABAergic systems in a number of characteristic cannabinoid behaviors, including discriminative stimulus effects, hypothermia, and catalepsy [present study; (10,11,14)]. Although antagonist studies reveal that Δ^9-THC's cannabinoid effects are clearly mediated by action at cannabinoid CB1 receptors, those of the benzodiazepines appear to be related to their action at GABA-associated benzodiazepine sites. Despite these separate neural mechanisms, however, the discriminative stimulus effects of high doses of benzodiazepines may partly resemble the intoxicating effects of marijuana. Although the current controversy surrounding the issue of the medical use of marijuana has fo-

cused primarily on beneficial and harmful effects to patients of marijuana alone, the results of the present study suggest that further investigation of the potential for increased marijuana-like intoxication and impairment of those patients who may already be receiving benzodiazepines for their medical condition seems warranted.

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