Life Sciences, Vol. 38, pp. 1581-1589 Pergamon Press
Printed in the U.S.A.

THE ROLE OF BENZODIAZEPINE RECEPTORS IN THE DISCRIMINATIVE STIMULUS PROPERTIES OF DELTA-9-TETRAHYDROCANNABINOL

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(Received in final form February 12, 1986)

Summary

Twenty male Sprague-Dawley rats were trained to discriminate 3.0 mg/kg delta-9-tetrahydrocannabinol (THC) from its vehicle. Following acquisition of this discrimination animals were tested for generalization to 3.0 mg/kg diazepam. Thirteen animals showed a generalization from THC to diazepam, whereas the remaining seven animals did not. The generalization curve for diazepam was dose-dependent from 0.1 to 10.0 mg/kg in the first group; the latter group showed no generalization from THC at any dose of diazepam in this range. No differences were found between these groups in the generalization curve for THC. The benzodiazepine antagonist Ro 15-1788 (2.0 mg/kg) antagonized the generalization to diazepam in the group that discriminated diazepam as THC. In contrast, Ro 15-1788 increased THC lever responding of 10 mg/kg diazepam in the group which did not generalize from THC. Ro 15-1788 did not alter the discriminability of THC in either group. THC also showed partial generalization to pentobarbital (1 to 10 mg/kg). The generalization was again complete in one subgroup and absent in another, but there was only a 43 percent overlap between the subgroups found with testing for generalization to diazepam. The percent THC lever responding with 3.0 mg/kg pentobarbital was increased by Ro 15-1788 in the group which generalized to diazepam, but not the other group. These data suggest that the discriminative stimulus properties of THC may have some commonality with the effects of diazepam in a subpopulation of rats trained to discriminate THC. These THC-like effects of diazepam are probably mediated by benzodiazepine receptors since they are antagonized by a specific benzodiazepine receptor antagonist.

Marihuana and delta-9-tetrahydrocannabinol (THC), the putative major psychoactive chemical in marihuana, have been reported to have effects similar to a number of classes of therapeutic drugs. Included among these effects are the anti-convulsant and anti-anxiety properties of THC (1). The cannabinoids cannabidiol and nabilone have also been reported in some paradigms to have anti-anxiety effects in animals and humans (2-4).

Despite the similarities between the cannabinoids and classical anti-convulsant and anti-anxiety agents, drug discrimination procedures have generally shown that animals do not generalize from the THC cue to the CNS depressants (5-8) or vice versa (6,9). While the authors of these papers conclude that cross-generalization does not occur between THC and diazepam or pentobarbital, the data are not so clear. Browne and Weissman (8) reported that 52 percent of animals administered diazepam generalize from THC to diazepam at

some dose. In addition, Bueno et al. (7) demonstrated that 50 percent of animals trained in a discrimination task transfer from THC to 15 or 20 mg/kg pentobarbital. Given the ambiguity of these findings, experiments were conducted to examine the discriminative stimulus properties of THC and the relationship of this cue to the stimulus properties of diazepam and pentobarbital.

Methods

Subjects. Twenty male Sprague-Dawley rats (Dominion Laboratories, Herndon, VA) were used. Animals were housed individually in stainless steel wire cages in animal quarters with controlled temperature, humidity and light cycle (0800-2000 light).

Training: Animals were food-deprived to 80% of their free-feeding weights. Diets were supplemented with additional food after behavioral sessions to maintain desired weights. Subjects were trained to press a lever for a reinforcer of sweetened milk. Animals were then trained in a two-lever operant discrimination of 3.0 mg/kg THC from the vehicle (1.5% emulphor:1.5% ethanol (v:v) in saline). The training procedure has been described in detail elsewhere (10). Responding on one lever was reinforced following administration of 3.0 mg/kg THC on a fixed ratio 10 (FR-10) schedule; responding on the other lever was reinforced on FR-10 after vehicle injection. Both THC and the vehicle were administered i.p. 30 min prior to the beginning of the 10-min training session. The operant cages were standard Coulbourn modular chambers located in sound attenuating boxes. Rockwell Aim 65 microprocessors were used for programming and data collection.

Testing: After acquisition of the discrimination task animals were tested in a dose-response to THC. A criterion was set such that in order for an individual animal to be tested the animal must have exceeded 90% correct lever responses on the last previous THC and vehicle training days. Testing was done on Wednesdays and Saturdays with Mondays, Tuesdays, Thursdays and Fridays for training. Animals were tested for 2.5 min with both levers reinforced. The percent drug lever responding on the first FR-10, as well as the total session responding, were recorded. Overall response rates for the test session were also recorded. After a dose effect curve for THC had been completed, each animal was tested following administration of 3.0 mg/kg diazepam (15 min pretreatment) in the 1.5% emulphor:1.5% ethanol vehicle. The dose-response curve to diazepam was then completed in all animals. Furthermore, animals were tested for a dose-response to diazepam following co-administration of 2.0 mg/kg Ro 15-1788. This dose of Ro 15-1788 has been previously shown to be effective in blocking other behavioral effects of diazepam (11). Fifteen animals were subsequently tested for the generalization from THC to doses of 1, 3 and 10 mg/kg pentobarbital in a 1.5% emulphor:1.5%ethanol vehicle (15 min pretreatment). Pentobarbital (3.0 mg/kg) and THC (3.0 mg/kg) were also tested in combination with 2.0 mg/kg Ro 15-1788.

Drugs: THC was obtained from the National Institute on Drug Abuse (Bethesda, MD); diazepam and Ro 15-1788 were gifts from Hoffman-LaRoche Laboratories (Nutley, NJ): and Na pentobarbital was obtained from Sigma Chemical Co. (St. Louis, MO). Doses of THC, diazepam and Ro 15-1788 refer to the weight of the free base, whereas the doses of Na pentobarbital refer to the weight of the salt. THC, diazepam and NA pentobarbital were placed into a vehicle consisting of a solution of 1.5% emulphor:1.5% ethanol: 97% saline (v:v:v); Ro 15-1788 was suspended in a solution of 0.5% methylcellulose. All drugs were injected i.p. in a volume of 1 ml/kg.

Statistics. Dose response curves for diazepam, THC and pentobarbital were compared between responders and non-responders using a two-way analysis of variance. Percentage data were transformed using the angular transformation. Individual comparisons were made using a least significant differences (lsd) test (12). The effects of Ro 15-1788 on the discrimination of THC, diazepam or pentobarbital were tested using a two-way ANOVA with lsd test for individual differences. Criterion for statistical significance was set a $p < 0.05$ for all tests.

Results

Overall, following acquisition of the discrimination of THC (3 mg/kg) from vehicle animals made 99 percent of their responses on the drug lever when administered 3 mg/kg THC and 12 percent of their responses on the drug lever following administration of vehicle (Table 1). Administration of 3 mg/kg diazepam resulted in a overall partial generalization from THC with animals emitting 64 percent of their responses on the drug lever. Similar results were obtained when 10 mg/kg pentobarbital was administered (Table 1).

TABLE I

Effects of Diazepam and Pentobarbital
in Animals Trained to Discriminate THC from Vehicle

Drug	Dose	N	%DLR[1]	RR (resp/sec)
THC	3 mg/kg	19	99±1**	.99 ± .11
VEHICLE	----	19	12±7*	.83 ± .07
DIAZEPAM	3 mg/kg	20	64±10*,**	.63 ± .08*
PENTOBARBITAL	10 mg/kg	13	74±10*,**	.39 ± .08*,**

Data represents mean ± 1 S.E.M. of the diazepam-responder and diazepam-non-responder groups combined. 1- Percent drug-lever responding. * significantly different from THC, ** significantly different from vehicle, ANOVA, lsd test, $p < 0.05$.

Closer examination of the data, however, shows that of the 20 animals tested in this experiment 13 animals made over 90% of their responses on the THC lever after administration of 3.0 mg/kg diazepam; the remaining seven animals produced only 14 percent THC lever responding following this dose of diazepam (Fig. 1). When the animals in the first group (diazepam-responders) were tested with a full range of doses of diazepam a standard dose-effect curve was generated. The latter group (diazepam-non-responders), however, did not generalize to any dose of diazepam. Furthermore, diazepam (10 mg/kg) produced a greater suppression of responding in the diazepam-non-responders than in the diazepam-responders (Fig. 1). The discriminability of diazepam as THC in the diazepam-responder group was significantly antagonized by Ro 15-1788 (Fig. 1; $F(1,107) = 4.71$). The effects of 1.0 and 3.0 mg/kg diazepam were significantly antagonized by co-administration of Ro 15-1788 (lsd test). Ro 15-1788 potentiated the discrimination of diazepam as THC in the diazepam-non-responder group ($F(1,51)=4.70$, $p < 0.05$; Fig. 1).

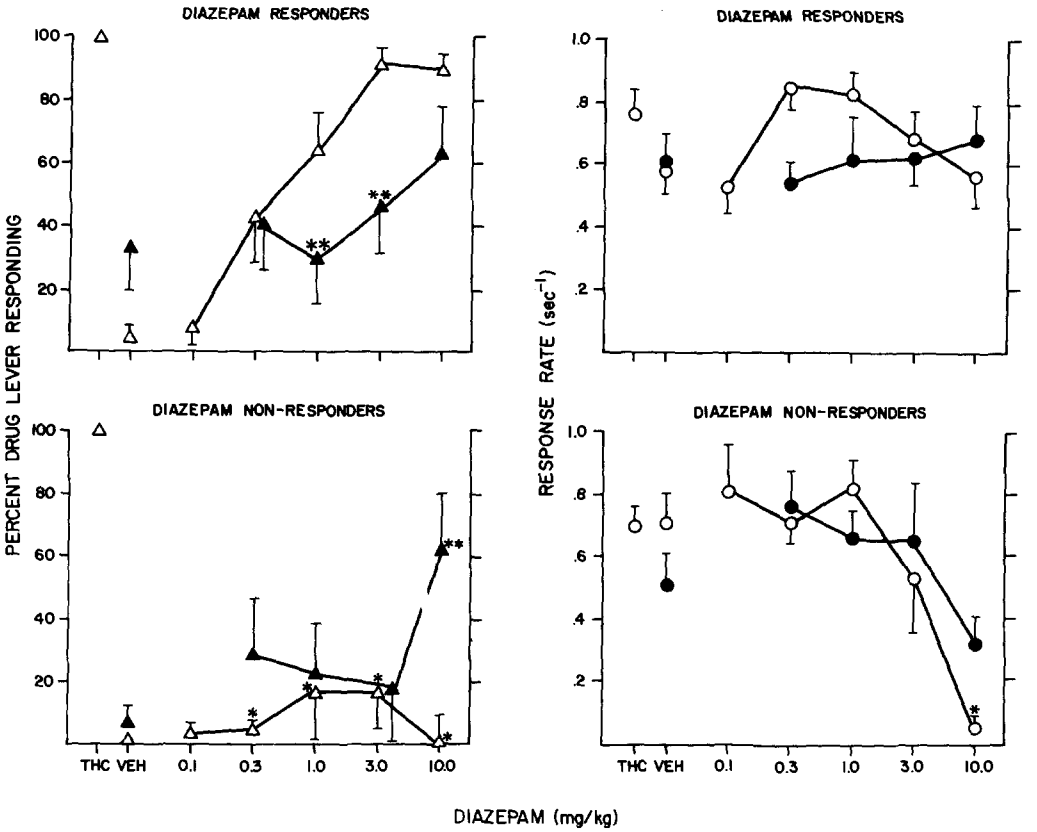

FIG. 1

Generalization curves for diazepam in two groups of rats trained to discriminate THC from vehicle. Left panels show the percent drug lever responding in diazepam-responders (top) and diazepam-non-responders (bottom). Right panels shows response rates for the two groups. Open symbols represent the effects of diazepam alone; closed symbols represent the effects of diazepam in combination with 2.0 mg/kg Ro 15-1788. * = Significantly different from diazepam-responders group. ** = Significantly different from diazepam alone, two-way analysis of variance, lsd test, $p < 0.05$.

Retrospective analysis of the ability of the two groups to discriminate THC from saline showed that no differences exist between groups in the dose-response curve for THC in this discrimination (Fig. 2; $F(1,17)=3.08$). Pretreatment of the animals in either group with Ro 15-1788 (2.0 mg/kg) did not affect the animals ability to discriminate the training dose of THC from the vehicle (Table II; $F(1,7)=1.98$), but did produce a significant suppression of response rates ($F(1,7)=18.84$, $p < 0.01$).

The discrimination of pentobarbital from vehicle did not differ in either group (Fig. 3; $F(1,35)=.091$). Both groups showed a partial generalization from THC to pentobarbital, with 10.0 mg/kg pentobarbital producing 65 percent drug lever responding in the diazepam-responder group and 84 percent drug lever responding in the diazepam-non-responder group. Pretreatment with Ro 15-1788 produced a significant potentiation of the effect of 3.0 mg/kg pentobarbital in the diazepam-responder group (Table II; $F(1,12)=7.64$), $p < 0.05$).

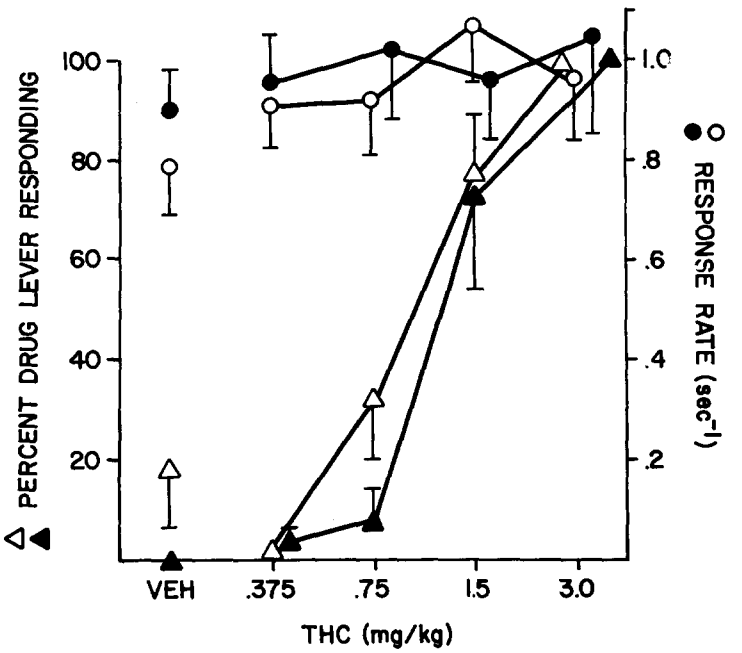

FIG. 2

Generalization curves for THC in diazepam-responders and diazepam-non-responders in animals trained to discriminate THC from vehicle (see text). Responders are represented by open symbols: non-responders are represented by closed symbols.

Discussion

When data from all animals are analyzed together, the results of these experiments agree overall with the results of previous experiments. Animals showed a partial generalization from THC to diazepam (Table I). A partial generalization to pentobarbital was also seen. These results are similar to those reported previously (7,8).

A subpopulation of rats, however, showed a dose-dependent generalization to diazepam (Fig. 1). This effect was attenuated by administration of the benzodiazepine antagonist Ro 15-1788. Herling and Shannon (13), Nielsen et al. (14) and Stephens et al. (15) have reported that Ro 15-1788 antagonizes the discriminative stimulus properties of diazepam. Ro 15-1788 alone generated a low level of drug lever responding that was greater than vehicle. This may be due to the partial agonist properties of Ro 15-1788, although these effects are generally seen only at much higher doses of Ro 15-1788 (16,17).

Retrospective analysis of the discrimination of THC in these two groups of animals demonstrates that there are no differences between the groups in their

Table II

Effect of Ro 15-1788 on the Discrimination of THC or Pentobarbital

Drug	Dose (mg/kg)	Diazepam-Responders		Diazepam-Non-Responders	
		%DLR [1]	RR [2]	%DLR	RR
Veh	---	4±4 (13)	.58±.07	0±0 (7)	.71±.09
Ro 15-1788	2.0	33±14 (11)	.61±.09	6±6 (7)	.51±.10
THC	3.0	98±2 (5)	1.05±.27	99±1 (4)	.98±.19
THC + Ro 15-1788	3.0	78±20 (5)	.38±.11*	93±7 (4)	.30±.04*
Pentobarb.	3.0	7±7 (8)	.78±.05	21±13 (6)	.80±.15
Pentobarb. + Ro 15-1788	3.0	65±14**(8)	.85±.11	31±20 (6)	.60±.18

1. Percent drug lever responding during 2.5 min test period. Data represents mean ± S.E.M.; number in parenthesis indicates n.
2. Response rate in responses/sec during 2.5 min test period. Data represents mean ± S.E.M.

* Significantly different from THC alone, two-way ANOVA, lsd test, $p < 0.05$

** Significantly different from pentobarbital alone, two-way ANOVA, lsd test, $p < 0.05$.

ability to discriminate THC from vehicle. Furthermore, Ro 15-1788 did not affect the discrimination of THC (Table II); although Ro 15-1788 in combination with THC produced a significant suppression of response rates in both groups. The generalization to diazepam is, therefore, not due to differences in the animals' ability to discriminate THC from its vehicle.

THC also partially generalized to pentobarbital. No differences were seen between the two groups of rats. Examination of the data, however, shows that, in the case of pentobarbital discrimination, animals could also be categorized into two groups, those that generalize completely to pentobarbital and those that do not. In the group that generalizes to diazepam, 4 of 8 animals generalized completely to 10.0 mg/kg pentobarbital, 2 animals did not generalize and the remaining 2 showed a partial generalization. In the diazepam-non-responder group 3 animals showed a complete generalization to 10.0 mg/kg pentobarbital, whereas the remaining two animals showed partial generalization from THC to pentobarbital. Thus, the two groups of animals (based on diazepam generalization) do not show a similar generalization to another drug with similar properties.

Ro 15-1788 potentiated the effects of 3.0 mg/kg pentobarbital in the responders but not the non-responders (Table II). This potentiation of the effects of pentobarbital has also been seen in other behavioral paradigms

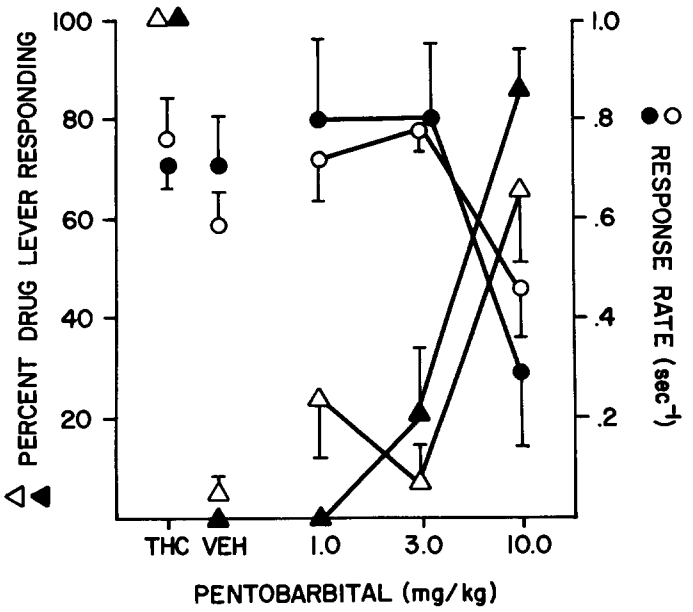

FIG. 3

Generalization curves for pentobarbital in diazepam-responders and diazepam-non-responders to diazepam in animals trained to discriminate THC from vehicle (see text). Responders are represented by open symbols: non-responders are represented by closed symbols.

(11,17). This further supports the partial agonist activity of Ro 15-1788 although other interactions with pentobarbital, i.e., absorption and/or metabolism, cannot be ruled out.

Differential responses in rats to the effects of benzodiazepines have also been reported in conflict procedures (19-21). Up to 30% of the animals in a conflict procedure do not show an anti-conflict response following administration of benzodiazepines; in the present study 35% of the animals do not generalize to THC. Although this may be coincidental, it may be hypothesized that the responders and non-responders to benzodiazepines in conflict procedures may be the same populations found in the discrimination generalization to THC. This would further suggest that the discriminative stimulus properties of THC which generalize to diazepam may relate to the possible anxiolytic properties of THC.

Colpaert et al. (22) have recently shown that the "partial" generalization of fentanyl to morphine is due to 100% generalization to morphine in some rats whereas others showed little or no generalization. These findings suggest that the "cue" produced by drugs may have multiple components and that the drug cue may not be unitary. A given animal may attend to a particular portion of the cue whereas another animal may relate to different aspects of the drug cue. This hypothesis receives further support from the present data of the

interaction of Ro 15-1788 with diazepam in the two groups. Whereas co-administration of the benzodiazepine antagonist to the rats which generalized from THC to diazepam reduced drug lever responding, Ro 15-1788 increased drug lever responding in the diazepam-non-responder group. This was accompanied by a reversal by Ro 15-1788 of the rate decreasing effects of diazepam in the diazepam-non-responders. Thus, Ro 15-1788 may be unmasking the relevant stimulus cues of diazepam in the diazepam-non-responder group, while blocking the cues in the diazepam-responder group. Furthermore, the cues which the diazepam-non-responder group attend to do not involve the benzodiazepine receptor blocked by Ro 15-1788.

Previous work has reported the anxiolytic and anticonvulsant properties of cannabinoids (1-4). The anxiolytic properties of cannabinoids, however, are not consistent in all subjects. In fact, the primary toxic effect of THC and marihuana is acute anxiety (1). Thus, the properties of THC in relation to anxiety states varies in human populations. The heterogeneity of drug responses in human populations is a well accepted fact; this is less widely accepted in experimental animal studies.

Acknowledgements

This work was supported by National Institute on Drug Abuse grant DA-00490. D. J. Mokler was supported by the National Institute on Drug Abuse Training Grant DA-07027.

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