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Evidence for separate neuronal mechanisms for the discriminative stimulus and catalepsy induced by Δ^9-THC in the rat

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Abstract. The cataleptogenic effect of Δ^9-THC was compared to its discriminative stimulus effects in rats. The ED_{50}s for the discriminative stimulus and catalepsy were 0.8 and 4.0 mg/kg, respectively, while their time courses were very similar. The ED_{50} of Δ^9-THC for catalepsy in experimentally naive rats was not different from that in rats trained with the drug discrimination procedure, indicating that the cataleptogenic effect was not appreciably attenuated by long-term exposure to low doses of Δ^9-THC. Pharmacologically, the catalepsy produced by Δ^9-THC more closely resembled that of haloperidol than of morphine, since anticholinergic pretreatment eliminated the Δ^9-THC-induced catalepsy while pre-treatment with naloxone had no effect. Although the cataleptogenic effect of Δ^9-THC could be pharmacologically manipulated by anticholinergic pre-treatment, its discriminative stimulus effects were not changed in the same animals. These results demonstrate that distinctive mechanisms of action exist for these cannabinoid-induced behaviors.

Key words: Discriminative stimulus – Catalepsy – Marijuana – Δ^9-Tetrahydrocannabinol – Opiate – Neuroleptic – Rats

The myriad effects ascribed to the constituents of cannabis, most specifically Δ^9-tetrahydrocannabinol (Δ^9-THC), range from the well described psychoactivity to numerous pharmacological effects such as analgesia, anti-emetic and anti-convulsant effects. Since the behavioral effects of the cannabinoids are unique to this class of compounds, one would expect that the mechanisms responsible for these effects would be distinguishable from those mediating the behavioral effects of other centrally acting drugs. However, the central mechanisms that are involved in the actions of the cannabinoids remain ill-defined.

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While several behavioral measures in laboratory animals may be used to evaluate the actions of the cannabinoids, the drug discrimination paradigm has proven to be an excellent predictor of psychoactivity (Weissman 1978; Ford et al. 1984). It is also noteworthy that many compounds have been tested as putative blockers of the Δ^9-THC discriminative stimulus, but there have been no reports of any successful antagonists (Browne and Weissman 1981). Catalepsy, on the other hand, is a behavior produced in several species by cannabinoids as well as many drugs. It has been especially well characterized in rodents as an effect of opiates and neuroleptics. In these two drug classes, catalepsy has been shown to be a pharmacologically distinguishable behavioral effect, which is mediated by distinct neural pathways and mechanisms (Kuschinsky and Hornykeiwicz 1972; Ezrin-Waters et al. 1976; Barghon et al. 1981; Fujiwara et al. 1985; Broekkamp et al. 1988; Consolo et al. 1988). Although opiate-induced and neuroleptic-induced catalepsy are distinguishable in many respects, their responses to specific antagonists most directly demonstrate the involvement of distinctly different mechanisms. The catalepsy caused by morphine is blocked by the classical opiate antagonist naloxone, while that caused by neuroleptics is not (Kuschinsky and Hornykeiwicz 1972). Further, the catalepsy of haloperidol is decreased by anticholinergics, while that of morphine is not (Costall and Naylor 1974; Ezrin-Waters et al. 1976). Although apomorphine, a D_1–D_2 dopaminergic agonist, decreases the catalepsy caused by both prototypic drugs, it appears to be much more effective in reducing the opiate rather than the neuroleptic catalepsy (Ezrin-Waters et al. 1976).

Cannabinoids have also long been known to cause catalepsy in animals (Loewe 1946), and this effect has been the target of many studies. In brief, manipulations of systems such as the serotonergic (Fujiwara et al. 1985), cholinergic (Ukei 1980; Moss et al. 1987), adrenergic (Fujiwara et al. 1985; Kataoka et al. 1987), as well as prostaglandins (Coupar and Taylor 1982; Ono et al. 1986) have all been implicated in having an effect on THC-induced catalepsy in rats. All studies seem to in-

dicate cannabinoid catalepsy is a primary central effect with the exception of one recent report in mice (Burstein et al. 1989).

The present study was designed to investigate whether the catalepsy induced by Δ^9-THC in rats is mediated by mechanisms distinct from those of opiates or neuroleptics. Further, our aim was to determine if Δ^9-THC's catalepsy and discriminative stimuli are mediated by similar mechanisms.

Materials and methods

Subjects. Male Sprague Dawley rats (Dominion Labs, Dublin, VA) were used for all experiments. The animals were individually housed in an animal room with a normal 12 h light/dark cycle and an ambient temperature of 20–22° C. Animals used in acute experiments for catalepsy determination weighed 250–300 g upon arrival. Food and water were continuously available, and the animals were tested within 3 weeks of their arrival. Rats that were trained to discriminate Δ^9-THC had free access to water but were maintained at approximately 300 g body weight by controlled feeding.

Apparatus. Eight identical, two-lever operant chambers (Lafayette Instrument Co., Lafayette, IN) were used for the drug discrimination component of the experiment. They were each housed in a sound attenuating chamber and ventilated by an exhaust fan, which also provided white noise during the sessions. The two response levers were located on the front panel of these chambers and separated by a food tray into which the reinforcement was delivered by means of an automated pellet feeder. The reinforcements consisted of 45 mg Bio-Serve™ rodent chow pellets (Frenchtown, NJ). Each lever was illuminated by a 4 W light, which served as an exteroceptive cue to signal the start of a session. Two Commodore 64™ micro-computers controlled reinforcement contingencies and recorded the data.

Catalepsy was quantified by an adaptation of the mouse ring test (Pertwee 1972). Rats were placed on a wire ring (13 cm in diameter) with a wooden backboard (40 cm in diameter) attached to a ring stand approximately 60 cm above the bench top. Experienced observers, using a stopwatch, recorded the time during a 5-min period in which the animal remained in a motionless or catatonic state. The catalepsy tests were conducted 30 min from the time of injection for all but the time course experiments. Two rats were evaluated simultaneously by one observer who was not informed of the treatment the animals had received. Results were recorded as seconds of immobility (maximum of 300).

Drugs. Δ^9-THC was obtained from the National Institute on Drug Abuse (NIDA) and dissolved in a 1:1 (v:v) mixture of absolute ethanol and Emulphor™ (GAF Corp., Linden, NJ) so that the concentration was 100 mg Δ^9-THC/ml. Final drug concentrations were prepared daily by adding saline to this stock to form a clear, homogeneous suspension of emulphor:ethanol:saline (1:1:18). The injection volume for all drugs was 1 ml/kg body weight. Vehicle injections consisted of the emulphor:ethanol:saline (1:1:18). In order to administer a 30 mg/kg dose of Δ^9-THC, it was necessary to adjust the vehicle to 1:1:4. When this vehicle was tested separately in the discrimination colony, the drug-lever responses were found not to differ significantly from those obtained with the 1:1:18 vehicle (data not shown).

The haloperidol was purchased from LyphoMed™ (Rosemont, IL). The concentration of this commercial solution was adjusted with distilled water/lactic acid (pH 3.0). The morphine sulfate (NIDA) was prepared in the same vehicle used for the cannabinoids.

Naloxone HCl, apomorphine HCl, atropine SO_4, scopolamine methylbromide (MBr), and scopolamine HCl were purchased from Sigma Chemicals (St Louis, MO) and, with the exception of apomorphine, were prepared using the cannabinoid vehicle (1:1:18). The apomorphine HCl required the addition of 0.2 mg/ml ascorbic acid and 0.25% butanol to the emulphor/ethanol (1:1) prior to the addition of saline. All drugs were prepared on the day of their use, and doses are expressed in terms of the salt where appropriate.

Drug discrimination training. Animals were trained to discriminate between an IP injection of Δ^9-THC (3 mg/kg) and vehicle, given 30 min before being placed in the operant chambers. The protocol used for the training and testing of the discriminators generally followed established two-lever operant procedures (Weissman 1978; Järbe and McMillan 1979; Ford et al. 1984; Martin et al. 1984). Briefly, the animals were trained once a day in 10-min sessions to respond on one of the two levers under a fixed ratio 10 (FR10) schedule of reinforcement for a food reward, i.e., after 10 successive presses on the correct lever. The correct lever was determined by the preceding injection. During training sessions, only responses on the drug lever were reinforced after an injection of Δ^9-THC at 3 mg/kg (1 ml/kg.). The opposite lever was reinforced after an injection of the vehicle. Responses on the incorrect lever had the consequence of resetting the counter of the correct lever to zero and requiring ten consecutive correct responses before a reward was delivered. In order to speed the acquisition of the discrimination, both levers as well as the differential stimulus were presented from the outset of training as the rats progressed from a continuous reinforcement schedule (FR1) to the FR10 (Overton 1979). Drug (D) and vehicle (V) training days were scheduled on a double alternation sequence (DDVVDD...). In order to control for a possible lever bias, the lever assignments were counterbalanced across the colony. For half the colony, the left lever was paired with reward after Δ^9-THC, whereas right lever reinforcement was paired with the Δ^9-THC injection for the other half of the colony.

After approximately 25–30 successive pairings of drug or vehicle state with reinforcement on the appropriate lever, the rats reliably learned to discriminate between the two injections and consequently responded primarily (more than 80%) on the appropriate lever. The acquisition of the discrimination was monitored by observing the lever on which the first FR (FFR) was completed. When an animal performed at a rate of eight out of ten correct FFRs, it was considered eligible to be tested. The response rate (responses per second) was used as a measure of non-specific CNS depression.

Drug discrimination protocol. Test sessions differed from training sessions in that they were only 2 min long and completed FRs on either lever were reinforced. Stimulus control was assessed by performance on "check" sessions, which consisted of test sessions preceded by an injection of one of the two training conditions (Δ^9-THC 3 mg/kg or vehicle) with the requirements that the group average was more than 80% drug-lever responding for Δ^9-THC and less than 20% drug-lever responding for a vehicle "check". A "check" session of each control condition preceded each set of experimental manipulations, i.e., dose-response curves or antagonist studies, and were used as the control points for that experiment. Stimulus control is even more dramatically shown by repetitive testing. In this testing protocol, the animals are injected with the vehicle and tested 30 min later. At the conclusion of that 2-min test session, they are removed from the operant chamber, injected with the training dose (3 mg/kg) of Δ^9-THC, and returned to their home cage. After 30 min they are retested when despite having been reinforced on the vehicle lever 30 min earlier, the animals predominantly switch to the opposite (Δ^9-THC) lever. The high degree of stimulus control demonstrated in these tests allowed for the time course experiments to be carried out in one test day, after a single injection. For this "repeat test" method of time course experiments, rats were tested at the time intervals indicated. Data collected from additional time course studies conducted with single tests at discrete time intervals between injection and test supported this method (data not shown, see also Järbe et al. 1981).

All test days were preceded by a minimum of 2 training days. Data from test sessions are presented as the percentage of total responses that were emitted on the drug lever as well as the response

rate. Typical dose-response relationships were obtained for both discriminable stimulus (drug cue) and response rate suppression and expressed as ED_{50}. The discriminative stimulus of Δ^9-THC has been shown to be pharmacologically specific in that the administration of non-related drug classes results in a preponderance of vehicle-lever responses (Weissman 1978; Browne and Weissman 1981).

Data analysis. The ED_{50} values were estimated by least squares regression analysis of the log dose-response relationship. An ANOVA with two-tailed Dunnett's t test statistic was used to compare group means of both discriminative stimulus and catalepsy data. Discriminative stimulus data for any particular test was included in the group means only if the animal had demonstrated stimulus control during the previous training day. This was determined according to the following criteria: FFR completed on the injection-appropriate lever, more than 80% responding occurred on that lever, and the response rate was greater than 0.05 responses per second. However, data from the unconditioned behaviors (response rate and catalepsy) of all animals were included in the group means.

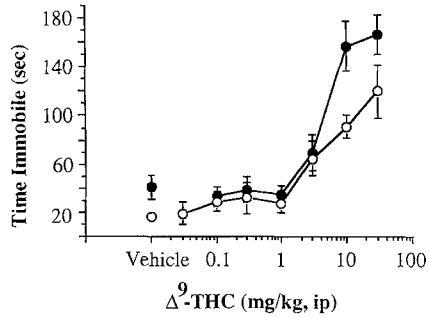

Fig. 1. Comparison of dose-response relationship of Δ^9-THC-induced catalepsy. Experimentally naive animals (●) were tested 30 min after an IP injection. The THC discriminators (○) were tested immediately following an operant test session at approximately 33 min after IP injection. Means ± SEM of immobility (s) were determined for a minimum of nine animals/group of naive rats and 22 animals/group of THC discriminators

Results

Comparison of catalepsy and discriminative stimulus properties of Δ^9-THC

A dose responsive state of immobility was observed in experimentally naive rats treated with Δ^9-THC when placed on the rings (Fig. 1). A maximum catelepsy of approximately 55% (approximately 165 seconds) was obtained with a dose of 30 mg/kg. The ED_{50} was determined to be 3.7 mg/kg.

The discriminative stimulus of Δ^9-THC was also dose responsive. The ED_{50} for the discriminative stimulus ranged from 0.6 to 1.0 mg/kg among five different groups of trained rats ($N=8$/group) used for the experiment, and the ED_{50} for suppression of response rate was between 8 and 11 mg/kg. Pooling all of these data yielded the plot shown in Fig. 2. ($N=40$) in which the ED_{50} for the total data set was 0.78 ± 0.09 for discrimination and 9.52 ± 0.48 mg/kg for response rates (mean ± SEM). Both the catalepsy and the discriminative stimulus displayed a very similar time course, which was approximately 80% maximal within 30 min of injection and peaked by 60 min (Fig. 3). Then, % drug choice declined slightly, but remained around 60% for up to 6 h, whereas catalepsy scores approximated vehicle levels after 6 h. Further studies have shown the % drug choice to decline to 30% by 8 h (data not shown).

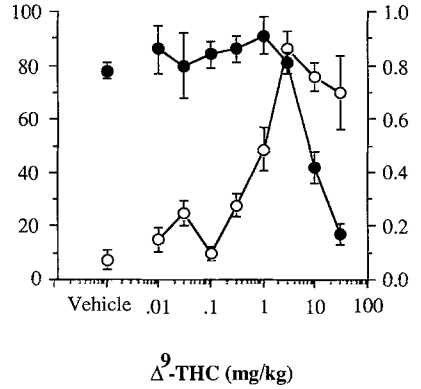

Fig. 2. The dose-response relationship of Δ^9-THC discriminative stimulus. Five groups of animals were injected with varying doses of Δ^9-THC ($N=40$). Combined group means ± SEM of the response rate (●) and % drug-lever responses (○) are plotted

Catalepsy measurement in rats trained to discriminate Δ^9-THC

In order to compare the cataleptic and the discriminative stimulus properties under similar conditions, both behaviors were measured in the same animal by placing the drug discrimination rats on the catalepsy test rings immediately after being tested in the operant chambers. The results demonstrated that even after receiving an average of three doses of Δ^9-THC (3 mg/kg) per week for more than a year, the animals still demonstrate a clear cataleptic effect to Δ^9-THC (Fig. 1). As expected from the cat-

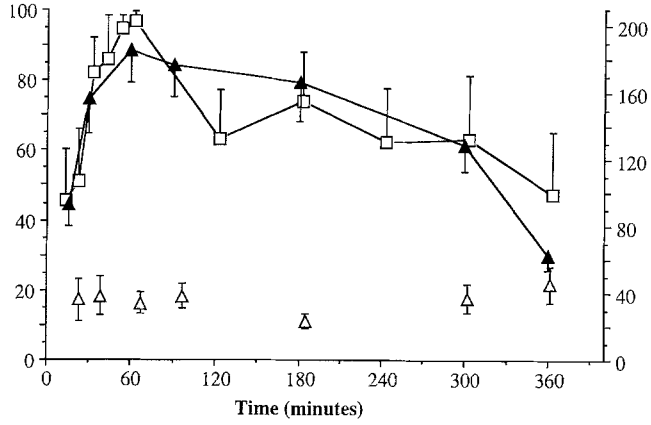

Fig. 3. Time course of Δ^9-THC-induced catalepsy and discriminative stimulus. Catalepsy (seconds of ring-immobility) was assessed in separate groups of naive rats administered a 10 mg/kg dose of Δ^9-THC (▲) or vehicle (△) $N=12$ rats/group except for the first and last two groups which consisted of 6 rats each. The time course of the discriminative stimulus was determined in one group of eight trained rats following a single administration of the training dose (3 mg/kg) of Δ^9-THC (□, % drug-lever response) using a repeated tests procedure. The means ± SEM, are presented for all measures. All animals were tested at the indicated times after IP injection

Table 1. Comparison of catalepsy induced by morphine, haloperidol, and THC

Treatment[a] (mg/kg, IP)	Seconds immobile (Mean ± SEM)		
Controls			
Naloxone 1	34.0 ± 11.0		
Haloperidol vehicle	12.0 ± 5.0		
Scopolamine 0.3	3.0 ± 2.0		
Atropine 10	8.0 ± 4.0		
Apomorphine 30	1.0 ± 1.0		
1:1:18 vehicle	17.0 ± 9.0		
Interactions			
Cataleptogen		+ Antagonist	
Morphine 10	110.0 ± 28.0[b]	Morphine 10 + naloxone 1	60.0 ± 11.0
Morphine 30	230.0 ± 41.0[b]	Morphine 30 + naloxone 1	52.0 ± 9.0
Haloperidol 1	213.0 ± 13.0[b]	Haloperidol 1 + scopolamine 0.3	98.0 ± 5.0
		Haloperidol 1 + atropine 10	114.0 ± 11.0[b, c]
		Haloperidol 1 + apomorphine 10	129.0 ± 35.0[b, c]
		Haloperidol 1 + apomorphine 30	54.0 ± 36.0
Haloperidol 3	254.0 ± 11.0[b]	Haloperidol 3 + scopolamine 0.3	129.0 ± 12.0[b, c]
		Haloperidol 3 + atropine 10	161.0 ± 29.0[b, c]
THC 10	156.0 ± 21.0[b]	THC 10 + naloxone 1	174.0 ± 6.0[b]
		THC 10 + scopolamine 0.3	15.0 ± 9.0
		THC 10 + atropine 10	6.0 ± 3.0
		THC 10 + apomorphine 30	153.0 ± 39.0[b]

[a] Rats were injected with cataleptogen 30 min prior to testing
Antagonists were given 10 min prior to the cataleptogen
Data are presented for a minimum of 6 rats per treatment

[b] $P < 0.05$ compared to control
[c] $P < 0.05$ compared to the cataleptogen alone

alepsy experiments performed with the experimentally naive rats, higher doses were required for production of catalepsy than for the discriminative stimulus. In this group of discriminators the ED_{50} for catalepsy was 4.4 mg/kg, while the ED_{50} for discriminative stimulus was 0.98 mg/kg (data not shown). When comparing the catalepsy of naive rats to that seen after drug discrimination straining, the Δ^9-THC effect in the latter appeared to be slightly attenuated at the two highest doses. However, the ED_{50}s were 3.6 and 4.4 mg/kg in the naive and discrimination trained rats, respectively.

Comparison of catalepsy induced by Δ^9-THC, morphine, and haloperidol

In order to determine whether the catalepsy induced by Δ^9-THC could be distinguished from that produced by other drugs, several agents were evaluated for their ability to modify the catalepsy induced by haloperidol, morphine, and Δ^9-THC in experimentally naive rats (Table 1). Morphine at doses of 10 and 30 mg/kg produced statistically significant catalepsy. The opiate antagonist naloxone effectively blocked the morphine-induced catalepsy when administered 10 min before. Naloxone itself failed to produce catalepsy. Haloperidol at doses of 1 and 3 mg/kg produced profound catalepsy. The dopaminergic agonist apomorphine produced a dose-dependent attenuation of the haloperidol-induced catalepsy. Additionally, the catalepsy of haloperidol was reduced by pre-treatment with the anticholinergics scopolamine and atropine. These agents were then evaluated under the same experimental conditions for their effects on catalepsy induced by 10 mg/kg Δ^9-THC. Scopolamine and atropine, which were effective in reducing the catalepsy of haloperidol, completely abolished the catalepsy seen with Δ^9-THC. However, neither naloxone nor apomorphine had any effect on Δ^9-THC-induced catalepsy.

Differential effects on the cataleptic and discriminative stimulus properties of Δ^9-THC

In order to determine whether the same mechanism was responsible for both catalepsy and the discriminative stimulus, naloxone, haloperidol, apomorphine, scopolamine, and scopolamine MBr were evaluated for their effect on these Δ^9-THC-induced behaviors in the same animal (Table 2). For possible alterations in the discriminative stimulus, the challenge drug was given 10 min prior to a dose of 1 mg/kg Δ^9-THC. Although this dose is one third of the training dose, it approximates the ED_{50} for the discriminative stimulus. Thus, the drug-lever response seen with this dose is usually quite variable. However, this dose provides optimal conditions to test drug interactions when it is not known if a leftward or rightward shift of the dose response curve will be produced.

As demonstrated by the dose-response curve of Δ^9-THC itself, the accuracy and/or reliability of drug discrimination results is directly related to the response rate. Typically, low response rates associated with high doses reflect the result of averaging the response rates of ani-

Table 2. Pharmacological interactions with the effects of THC on % drug lever selection, response rate, and catalepsy (mean ± SEM)

Treatment[a] (mg/kg, IP)	% Drug lever responding[b]	Responses/second[b]	Seconds immobile[c]
Vehicle	16.0 ± 14.0	0.85 ± 0.09	24.0 ± 6.4
Apomorphine 0.1	5.0 ± 1.5 ($N=5$)	0.43 ± 0.14	24.0 ± 11.0
Apomorphine 1	44.0 ± 31.4 ($N=2$)	0.08 ± 0.05[d]	9.0 ± 7.6
Apomorphine 10	1.0 ± 1.0 ($N=1$)	0.04 ± 0.03[d]	1.0 ± 1.1[d]
Vehicle	12.0 ± 10.6	0.60 ± 0.08	10.0 ± 4.0
THC 1	31.0 ± 14.5	0.77 ± 0.13	36.0 ± 11.0
Naloxone 1	10.0 ± 4.7	0.41 ± 0.09	11.1 ± 5.7
THC 1 + naloxone 1	28.0 ± 14.4	0.51 ± 0.07	51.0 ± 13.0
Vehicle	36.0 ± 20.3	0.60 ± 0.13	18.0 ± 3.8
THC 1	31.0 ± 14.5	0.77 ± 0.13	36.0 ± 11.0
Haloperidol 0.1	38.0 ± 18.1	0.92 ± 0.07	Not tested
THC 1 + haloperidol 0.1	66.0 ± 16.8	0.64 ± 0.11	24.0 ± 8.2
THC 1	31.0 ± 14.5	0.77 ± 0.13	36.0 ± 11.0
THC 1 + scopolamine 0.3	68.0 ± 16.0 ($N=5$)	0.65 ± 0.13	56.0 ± 11.0
THC 10	83.0 ± 16.5 ($N=4$)	0.53 ± 0.17	86.0 ± 17.8
THC 10 + scopolamine 0.3	77.0 ± 4.5	0.08 ± 0.04[d]	7.0 ± 2.4[d]
THC 30	28.0 ± 0 ($N=1$)	0.17 ± 0.15	124.0 ± 26.0
THC 30 + scopolamine 0.3	No responding	0.03 = 0.0 ($N=1$)	54.0 ± 15.0[d]
THC 1	39.0 ± 17.9	1.11 ± 0.06	14.0 ± 5.9
THC 1 + scopolamine 3	58.0 ± 14.0 ($N=5$)	0.08 ± 0.03[d]	1.0 ± 0.6
THC 1	39.0 ± 17.9	1.11 ± 0.06	14.0 ± 5.9
THC 1 + scopolamine MBr 1	67.0 ± 20.7	0.38 ± 0.07[d]	76.0 ± 24.0[d]
THC 10	83.0 ± 16.5	0.53 ± 0.17	86.0 ± 17.8
THC 10 + scopolamine MBr 10	99.0 ± 1.5 ($N=3$)	0.14 ± 0.09	91.0 ± 21.0

[a] Antagonists were injected 10 min prior to THC
[b] $N=6$–8, except where noted otherwise
[c] Measured 33–40 min after injection of THC
[d] $P<0.05$ compared to controls

mals whose behavior is completely disrupted (i.e., not lever pressing) with those animals who are still responding. Therefore in Table 2, only the animals that responded greater than 0.05 responses per second are included in the % drug-lever responding column and the N is noted. The difference between this number and the N reported for the two unconditioned measures (responses per second and seconds immobile) is the number of animals not responding sufficiently to complete one FR. The data shown in this table were collected from five separate groups of discriminators over an average of 15 days.

Naloxone tested alone at 1 mg/kg for possible agonist actions in the drug discrimination paradigm resulted in vehicle-lever responding (Table 2). When this dose of naloxone was administered prior to 1 mg/kg Δ^9-THC, the % drug-lever responding was comparable to that of 1 mg/kg Δ^9-THC alone. Naloxone failed to alter Δ^9-THC-induced catalepsy which was consistent with the findings in the experimentally naive rats.

Scopolamine exerted a similar effect on cannabinoid-induced catalepsy in both discriminating and naive rats. Scopolamine (0.3 mg/kg) abolished the cataleptogenic effects of 10 mg/kg Δ^9-THC and not surprisingly a dose of 3.0 mg/kg blocked the catalepsy produced by 1 mg/kg Δ^9-THC. Interestingly, the low dose of scopolamine appeared not to eliminate the catalepsy produced by 1 mg/kg Δ^9-THC. This effect of the lower dose of scopolamine seemed somewhat idiosyncratic in that it antagonized the catalepsy produced by the 10 mg/kg Δ^9-THC but not the 1 mg/kg Δ^9-THC. In general, the 1 mg/kg dose of Δ^9-THC produces only minimal or threshold levels of catalepsy in which antagonism in the form of a further reduction is less easily measured. Administration of a combination of scopolamine (0.3 mg/kg) and Δ^9-THC (10 mg/kg) produced a relatively high degree of drug-appropriate responding, although the response rate was severely depressed. A high dose of scopolamine (3 mg/kg) was then paired with a 1 mg/kg dose of Δ^9-THC to determine whether a reversal of the discriminative stimulus was possible. Again, the operant behavior was severely disrupted; however, those animals responding did so primarily on the drug lever. Finally, a 0.3 mg/kg dose of scopolamine was combined with the minimally cataleptogenic dose of 1 mg/kg Δ^9-THC in an attempt to maintain adequate response rates. Response rates were adequate, but there was no antagonism of the Δ^9-THC cue. Scopolamine MBr at 1 mg/kg, when combined with 1 mg/kg Δ^9-THC resulted in 67% drug lever responding, which was not significantly different from that produced by Δ^9-THC alone. When this dose of scopolamine was paired with a cataleptogenic dose of Δ^9-THC (10 mg/kg), the operant responding was disrupted and the animals displayed a level of catalepsy comparable to that obtained with Δ^9-THC alone.

Apomorphine was also tested for THC-like discriminative stimulus properties. The lowest dose (0.1 mg/kg) resulted in obvious vehicle-lever selection, although there was some rate suppression. At a 10-fold higher dose the animals were severely disrupted, but there was evidence

of some drug-lever responding in two rats. This modest drug-lever responding produced by apomorphine prompted the testing of a higher dose which was found to completely inhibit responding. Apomorphine and scopolamine were unique in that their response rate effects were coupled with parallel changes in the catalepsy times of the rats. Haloperidol (0.1 mg/kg) neither substituted for Δ^9-THC nor prevented the perception of its cue when tested as an antagonist. Interestingly, the combination of inactive doses of haloperidol and Δ^9-THC failed to result in a level of catalepsy significantly different from that of vehicle.

Discussion

The potency of Δ^9-THC in our drug discrimination colony agrees well with published results from other two-lever operant paradigms (Weissman 1978; Browne and Weissman 1981). The time course for Δ^9-THC's cue was somewhat longer than that reported by Järbe et al. (1981). The initial decline in drug-lever responding reported at 2 h in that study was also apparent in our animals. However, our rats demonstrated an intermediate level of drug-appropriate responses for approximately 6 h after the injection, whereas Järbe et al. (1981) found that Δ^9-THC-treated animals were responding almost exclusively on the vehicle lever by 4 h. The longer time course found in the present study using the repeated test procedure (Järbe et al. 1981) was observed after single injections of Δ^9-THC as well (data not shown) and therefore was probably not a function of the repeated test procedure itself. The difference in time course between studies could be due to several factors which include animal strains, type of reinforcement and test duration.

The cataleptic response of rats to Δ^9-THC was dose related, and the potency of Δ^9-THC was in general agreement with previously published values (Ukei 1980). Furthermore, this response was not significantly altered by long-term treatment of rats with Δ^9-THC as evidenced by the catalepsy seen in the drug discriminating colony. Likewise, tolerance did not appear to develop to the discriminative stimulus. Both in our laboratory as well as several reports in the literature, rats have been used long term without an appreciable effect on the potency or efficacy of Δ^9-THC as a discriminative stimulus. It must be pointed out that studies which have addressed the question of cannabinoid tolerance utilized higher doses, and the schedule of administration was not intermittent such as it typically is for drug discrimination colonies.

As with the time course of the discriminative stimulus, the duration of the catalepsy observed in this study differs from literature values. Fernandes et al. (1974) reported that Δ^9-THC-induced catalepsy peaked at 2 h and returned to control levels by 4 h. This time course is substantially different from that observed in the present study, especially since the dose of Δ^9-THC used was twice that used here. However, the studies differ in that Fernandes et al. conducted catalepsy testing during the dark phase of the animal's light/dark cycle and they employed the "bar test" rather than the "ring test". Habituation or a practice effect to the ring test would not be an explanation of the longer time course of this study, since separate groups of naive rats were used for each time point.

The profile of Δ^9-THC catalepsy more closely resembled that of haloperidol than that of morphine. Firstly, the anticholinergics such as scopolamine and atropine reduced both Δ^9-THC and haloperidol catalepsy. These agents have been reported to be ineffective against the catalepsy of opiates (Kuschinsky and Hornykeiwicz 1972; Costall and Naylor 1974; Ezrin-Waters et al. 1976). Secondly, the dopaminergic agonist apomorphine had no effect on Δ^9-THC-induced catalepsy up to the highest dose tested (30 mg/kg). Ezrin-Waters et al. (1976) reported that apomorphine abolished the catalepsy of morphine with doses less than 10 mg/kg, while doses of 100 mg/kg were required to completely block that of haloperidol.

The direct comparison of the effect of various treatments on the discriminative stimulus and catalepsy of Δ^9-THC in the same animals reveals that while catalepsy can be pharmacologically manipulated by scopolamine and apomorphine, the discriminative stimulus cannot. Notably only one of these behaviors is associated with reinforcement while the other is not, which may have important behavioral consequences. It is known with regard to the development of tolerance for example, that the reinforcement density hypothesis predicts tolerance will develop more easily to the rate disrupting effects of a drug when this effect interferes with obtaining reinforcement (Schuster et al. 1966; Ferraro 1978). The result of pre-treatment with scopolamine on the discriminative stimulus is in keeping with the results previously reported for atropine (Browne and Weissman 1981). To our knowledge scopolamine has not been tested in a THC binding assay, however, many in vitro interactions of THC with the cholinergic system are known (Friedman et al. 1976; Dewey 1986). Haloperidol and naloxone have also been reported to be devoid of Δ^9-THC discriminative stimulus blocking effects (Browne and Weissman 1981). The testing of apomorphine for agonist activity extends the results of Bueno et al. (1976) to the two-lever food-reinforced operant paradigm.

Intuitively, one would expect that a decrease in response rate under operant conditions might be due to a central depressive effect of the treatment. Indeed, the pattern seen with Δ^9-THC itself is in keeping with this assumption. As the dose is increased the response rate decreases and the catalepsy times increase indicating more pronounced immobility. However, the results obtained with apomorphine and scopolamine suggest that a decrease in operant responding does not necessarily imply a general depressant action on all central functions. The discriminators tested with these drugs had catalepsy times comparable to vehicle-treated rats and yet were not responding in the operant chambers.

Finally, the results with scopolamine MBr indicated that this anticholinergic, which does not readily cross the blood-brain barrier, was unable to attenuate the discriminative stimulus of Δ^9-THC or its cataleptogenic properties. It was not surprising that the peripherally acting anticholinergic did not affect the discriminative stimulus in light of the fact that the centrally acting compound had no effect. However, the lack of effect on catalepsy indicated this behavior was also centrally mediated. The idea that drugs act on the central nervous system to produce catalepsy is supported by numerous studies in which the catalepsy produced by of Δ^9-THC and other drugs in rats has been induced by intracerebral routes of administration, as well as altered by brain lesions (Gough and Olley 1977, 1978; Ukei 1980; Kataoka et al. 1987). Recently, Burstein et al. (1989) proposed a peripheral mechanism of action for the cataleptogenic effect of cannabinoids in mice which involved the eicosenoid prostaglandins. While the findings with scopolamine MBr support the notion that the cannabinoids are acting directly on brain, the possibility that a peripheral mechanism exists, which scopolamine MBr does not block, has not been excluded. With respect to the discriminative stimulus however, Browne and Weissman (1981) have reported that the pre-treatment of their rats with aspirin, a prostaglandin synthesis inhibitor, did not block the Δ^9-THC discriminative stimulus.

Although cannabinoid receptors have been hypothesized to exist, it has only been recently that a cannabinoid binding site has been identified (Devane et al. 1988; Herkenham 1990) and cloned (Matsuda et al. 1990). Efforts designed to determine the functional significance of this binding site are complicated by the multitude of centrally mediated cannabinoid effects. Consequently, evidence of cannabinoid effects responding distinctly to pharmacologic manipulations aid in the elucidation of mechanism of action. The results reported here show that the discriminative stimulus and catalepsy caused by Δ^9-THC are mediated by two distinct mechanisms.

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References

Barghon R, Protais P, Colboc O, Costentin J (1981) Hypokinesia in mice and catalepsy in rats elicited by morphine associated with antidopaminergic agents, including atypical neuroleptics. Neurosci Lett 27:69–73

Broekkamp CL, Oosterloo SK, Berendsen HH, van Delft AM (1988) Effect of metergoline, fenfluramine, and 8-OHDPAT on catalepsy induced by haloperidol or morphine. Naunyn Schmiedebergs Arch Pharmacol 338:191–195

Browne RG, Weissman A (1981) Discriminative stimulus properties of Δ^9-tetrahydrocannabinol: mechanistic studies. J Clin Pharmacol 21:227S–234S

Bueno OFA, Carlini EA, Finkelfarb E, Suzuki J (1976) Δ^9-THC, ethanol and amphetamine as discriminative stimuli-generalization tests with other drugs. Psychopharmacology 46:235–243

Burstein SH, Hull K, Hunter SA, Shilstone J (1989) Immunization against prostaglandins reduces delta 1-tetrahydrocannabinol-induced catalepsy in mice. Mol Pharmacol 35:6–9

Consolo S, Forloni G, Ladinsky H, Palazzi E (1988) Enhancement of opioid cataleptic response by cortical frontal deafferentation or intrastriatal injection of NMDA-receptor antagonists. Brain Res 449:97–103

Costall B, Naylor RJ (1974) On catalepsy and catatonia and the predictability of the catalepsy test for neuroleptic activity. Psychopharmacology 34:233–244

Coupar IM, Taylor DA (1982) Alteration in the level of endogenous hypothalamic prostaglandins induced by Δ^9-tetrahydrocannabinol in the rat. Br J Pharmacol 76:115–119

Devane WA, Dysarz FA, Johnson MR, Melvin LS, Howlett AC (1988) Determination and characterization of a cannabinoid receptor in rat brain. Mol Pharmacol 34:605–613

Dewey, WL (1986) Cannabinoid pharmacology. Pharmacol Rev 38:151–177

Ezrin-Waters C, Muller P, Seeman P (1976) Catalepsy induced by morphine or haloperidol: effects of apomorphine and anticholinergic drugs. Can J Physiol Pharmacol 54:516–519

Fernandes M, Schabarek A, Coper H, Hill R (1974) Modification of Δ^9-THC-actions by cannabinol and cannabidiol in the rat. Psychopharmacology 38:329–338

Ferraro DP (1978) Behavioral tolerance to marihuana. In: Krasnegor NA (ed) Behavioral tolerance: research and treatment implication (NIDA Monogr, vol 18). Govt Printing Office, Washington, DC, pp 103–117

Ford RD, Balster RL, Dewey WL, Rosecrans JA, Harris LS (1984) The discriminative stimulus properties of Δ^9-THC: generalization to some metabolites and congeners. In: Agurell S, Dewey WL, Willette RE (eds) The cannabinoids: chemical, pharmacologic, and therapeutic aspects. Academic Press, New York, pp 545–561

Friedman E, Hanin, I, Gershon S (1976) Effect of tetrahydrocannabinols on ^{3}H-acetylcholine biosynthesis in various rat brain slices. J Pharmacol Exp Ther 196:339–345

Fujiwara M, Sakurai Y, Kiyota Y, Shimazoe T, Ohta H, Shibata S, Ueki S (1985) Behavioral pharmacology of amantadine with special references to the effect on abnormal behavior in mice and rats. Folia Pharmacologica Japonica 4:259–274

Gough AL, Olley JE (1977) Δ^9-Tetrahydrocannabinol and the extrapyramidal system. Psychopharmacology 54:87–99

Gough AL, Olley JE (1978) Catelepsy induced by intrastriatal injections of delta-9-THC and 11-OH-delta-9-THC in the rat. Neuropharmacology 17:137–144

Herkenham M, Lynn AB, Little MD, Johnson MR, Melvin LS, DeCosta BR, Rice KC (1990) Cannabinoid receptor localization in brain. Proc Natl Acad Sci USA 87:1932–1936

Järbe TUC, McMillan DE (1979) Discriminative stimulus properties of tetrahydrocannabinols and related drugs in rats and pigeons. Neuropharmacology 18:1023–1024

Järbe TUC, Swedberg MDB, Mechoulam R (1981) A repeated test procedure to assess onset and duration of the cue properties of (−)-Δ^9-THC, (−)-Δ^8-THC–DMH and (+)-Δ^8-THC. Psychopharmacology 75:152–157

Kataoka Y, Ohta H, Fujiwara M, Oishi R, Ueki S (1987) Noradrenergic involvement in catalepsy induced by delta 9-tetrahydrocannabinol. Neuropharmacology 26:55–60

Kuschinsky K, Hornykeiwicz O (1972) Morphine catalepsy in the rat: relation to striatal dopamine metabolism. Eur J Pharmacol 19:119–122

Loewe S (1946) Studies on the pharmacology and acute toxicity of compounds with marihuana activity. J Pharmacol Exp Ther 88:154–161

Martin BR, Kallman MJ, Kaempf GF, Harris LS, Dewey WL (1984) Pharmacological potency of R- and S-3′-hydroxy-Δ^9-tetrahydrocannabinol: additional structural requirement for cannabinoid activity. Pharmacol Biochem Behav 21:61–65

Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI (1990) Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 346:561–564

Moss DE, Manderscheid PE, Kobayashi H, Montgomery SP (1987) Evidence for the nicotinic cholinergic hypothesis of cannabinoid action within the central nervous system: extrapyramidal motor behaviors. In: Chesher G, Consroe P, Musty R (eds) Marihuana: an international research report. Australian Government Publishing Service, Melbourne, pp 359–364

Ono N, Saito R, Abiru T, Kamiya H, Furukawa T (1986) Possible involvement of prostaglandins in cataleptic behavior in rats. Pharmacol Biochem Behav 25:463–467

Overton DA (1979) Influence of shaping procedures and schedules of reinforcement on performance in the two-bar drug discrimination task: a methodological report. Psychopharmacology 65:291–298

Pertwee RJ (1972) The ring test: a quantitative method for assessing the "cataleptic" effect of cannabis in mice. Br J Pharmacol 46:753–763

Schuster CR, Dockens WS, Woods JH (1966) Behavioral variables affecting the development of amphetamine tolerance. Psychopharmacologia 9:170–182

Ukei S (1980) Abnormal behavior induced by Δ^9-tetrahydrocannabinol and its pharmacological characteristics. TIPS 1:126–129

Weissman A (1978) Generalization of the discriminative stimulus properties of Δ^9-THC to cannabinoids with therapeutic potential. In: Colpaert FC, Rosecrans JA (eds) Stimulus properties of drugs: ten years of progress. Elsevier, Amsterdam, North Holland, pp 99–122