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SR141716A Antagonizes the Disruptive Effects of Cannabinoid Ligands on Learning in Rats[1]

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ABSTRACT

The effects of cannabinoid ligands were studied in rats responding under a repeated acquisition procedure. Each session rats were required to learn a different three-response sequence; every third correct completion of the sequence resulted in the presentation of a food pellet. Errors produced a brief timeout but did not reset the chain. Neither injections of the centrally inactive cannabinoid, cannabidiol (3.2–100 mg/kg i.p.), nor the endogenous ligand, anandamide (0.01–18 mg/kg i.p.), affected rate or accuracy of responding. In contrast, Δ^9-tetrahydrocannabinol (3.2–18 mg/kg i.p.) and the long-acting analog of the endogenous ligand, R-methanandamide (1–18 mg/kg i.p.), produced dose-related increases in the total percentage of errors and decreases in the rate of responding. The brain cannabinoid receptor antagonist SR141716A (1–32 mg/kg) did not affect either accuracy or rate of responding when administered alone. A low dose of SR141716A (1 mg/kg), which had no effect when administered alone, antagonized the disruptive effects of Δ^9-tetrahydrocannabinol and R-methanandamide on rate and accuracy of responding and produced an estimated 3-fold shift to the right in the dose-effect curves. However, administration of SR141716A did not alter the effects of morphine. These results suggest that cannabinoid agonists produce disruptions of learning in rats through stimulation of the cannabinoid receptor. The data further suggest that whereas cannabimimetic agents can disrupt learning, the anandaminergic system may not be tonically involved in learning.

In the United States marijuana is the most widely used of all illicit drugs (Johnson *et al.*, 1993). In humans, disruption of short-term memory is a widely reported effect of Δ^9-THC (Miller and Branconnier, 1983). Despite a long history of human marijuana use, it is only in the last decade that we have started to understand the molecular basis for the behavioral effects of marijuana. Several recent findings have greatly advanced efforts to understand the behavior pharmacology of cannabinoids. These developments include the identification, cloning and expression of a selective cannabinoid receptor (Matsuda *et al.*, 1990), isolation of an endogenous cannabinoid ligand (Devane *et al.*, 1992) and most recently the synthesis of a selective antagonist, SR141716A [N-(piperidine-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide hydrochloride] (SR) (Rinaldi-Carmona *et al.*, 1994).

Receptor sequencing data has revealed that this receptor is part of the large family of G-protein coupled receptors. Quantitative receptor autoradiography using the cannabinoid receptor ligand ^{3}H-CP55,940 has shown that the central CB1 is regionally distributed within the brain with high concentrations in several areas. One of these sites of high concentration is the substantia nigra that is thought to play a role in the reinforcing properties of drugs (Herkenham *et al.*, 1990). Other areas of high cannabinoid receptor density also correlate with many known *in vivo* effects of Δ^9-THC. Receptor densities in the basal ganglia, cerebellum and the hippocampus parallel the *in vivo* effects of Δ^9-THC including, motor, cataleptic and amnestic effects, respectively. The endogenous ligand anandamide produces effects that are similar to those of Δ^9-THC in most assays of cannabimimetic activity. The duration of the effects produced by anandamide, however, are much shorter than those of Δ^9-THC. In rodents anandamide, like Δ^9-THC, has been reported to produce hypothermia and decrements in motor activity (Crawley *et al.*, 1993).However, unlike Δ^9-THC, anandamide did not disrupt memory in nonmatch-to-position tasks (Crawley *et al.*, 1993; Mallet and Beninger, 1996). Δ^9-THC produced both dose- and delay-dependent disruptions of performance, whereas anandamide was ineffective at all doses tested. Disruptions in the performance of this task, however, were produced when the administration of anandamide was preceded by the administration of PMSF (Mallet and Beninger, 1996). PMSF inhibits protease activity and thus extends the action of anandamide in rats. Another approach to extending the duration of action of anandamide has been to synthesize analogs that

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ABBREVIATIONS: Δ^9-THC, Δ^9-tetrahydrocannabinol; SR, SR141716A; CB, cannabinoid receptor, official designation for subtypes CB1 and CB2; PMSF, phenylmethylsulfonyl fluoride.

might be metabolized at a slower rate. This effort has produced R-methanandamide. Upon initial investigation this analog possesses a similar pharmacological profile to that of Δ^9-THC and anandamide, but has a substantially longer duration of action (Abadji *et al*., 1994). The behavioral effects of R-methanandamide closely parallel those of the cannabinoid agonists Δ^9-THC and anandamide (Romero *et al*. 1996), yet the effects of R-methanandamide on learning have not been reported.

Work with SR, the newly synthesized cannabinoid antagonist, has shown that this compound antagonizes the motor, hypothermic, and antinociceptive effects of cannabinoids (Rinaldi-Carmona *et al*., 1994). Mansbach *et al*. (1996) reported that SR reversed the disruptive effects of Δ^9-THC on the performance of a fixed consecutive number procedure and antagonized the effects of the cannabinoid agonist CP55,940 in an acoustic startle procedure. Drug discrimination studies have shown that SR is capable of reversing drug appropriate responding in pigeons, rats and monkeys trained to discriminate Δ^9-THC from saline (Mansbach *et al*., 1996; Wiley *et al*., 1995). In contrast to drug discrimination and physiological measures, the interactions of these agonists and antagonists have not been fully characterized in terms of their effects on learning.

In humans, disruption of short-term memory is a widely reported effect of Δ^9-THC (Miller and Branconnier, 1983). Similar deficits have been observed in animal models of learning and memory, most notably the eight-arm radial maze and the delayed matching to sample procedure. In the delayed matching to sample procedure Δ^9-THC, but not the non-centrally active cannabinoid cannabidiol, has been shown to produce alterations in hippocampal cell activity which are correlated with delay- and dose-dependent deficits (Heyser *et al*., 1993). Lictman *et al*. (1995), found that systemic administration of the cannabinoid agonists Δ^9-THC, WIN-55,212–2 and CP-55,940 disrupted behavior in an eight-arm radial maze procedure. The cannabinoid agonists increased the time required to finish the task and increased revisits to arms in which the pellet had already been consumed. Δ^9-THC was found to increase revisits more frequently than decreasing task completion time. These studies suggest that cannabinoid agonists act to disrupt measures of learning and memory. Compton *et al*. (1996) reported that Δ^9-THC and other cannabinoid agonists produced decreases in the locomotor activity of mice, although high doses of the antagonist SR alone increased locomotion. Given the reported amnestic effects of the cannabinoid agonists, the suggestion that SR may produces effects opposite to those of cannabinoid agonists raises the intriguing possibility that SR may enhance learning or memory when administered alone.

The aim of our study was to characterize the effects of the anandamide analog, R-methanandamide, Δ^9-THC and the antagonist SR on learning in a repeated acquisition procedure. Further, our aim was to determine if any of the disruptive effects of cannabinoids on learning are mediated by the CB1 receptor. The selectivity of Δ^9-THC and SR actions were addressed, respectively, by testing the non-centrally active cannabinoid, cannabidiol, and the active non-cannabinoid morphine. The technique of repeated acquisition was designed to test the effects of drugs on the acquisition of a discrimination. This technique has been used to characterize many different drug classes in a variety of species (Moerschbaecher and Thompson, 1980, 1983 and Pollard *et al*., 1981). In general, drugs that disrupt human cognition also disrupt learning in repeated acquisition tasks. The purpose of the present study was therefore to characterize the effects of the cannabinoid agonists, both alone and in combination with the antagonist SR, and investigate the potential nootropic effects of SR alone.

Materials and Methods

Subjects. Eighteen adult male Long-Evans rats (Charles River, Wilmington, MA) served as subjects. Each rat was maintained at a restricted weight of 80–90% (310–380 g) of its free-feeding weight. The free-feeding weight was measured several times per year and determined after two weeks of free access to food for each subject. Subjects were housed individually with free access to water on a light/dark cycle (lights on 6:00 A.M. to 6:00 P.M.). Animals were fed a diet of Purina rodent chow (St. Louis, MO) and Bio-Serv precision food pellets (45 mg) (Bio-Serv, Frenchtown, NJ). Pellets were received during daily sessions and rodent chow was provided one hour after each session.

Apparatus. For each session the subject was placed in a modified operant chamber (Coulbourn Instruments, Allentown, PA) 3 to 8 min. before the session started. Six identical experimental chambers were used for all experiments and each subject was always placed in the same chamber. Each chamber was located inside a light and sound attenuating enclosure equipped with fans to provide air circulation and masking noise. The front wall of each chamber was equipped with a house light (26 cm above the floor and centered), three translucent press-type key capable of being transilluminated with either red, white or amber colored light (8 cm apart, center to center and 10 cm above the floor), a pellet hopper with feeder light (1 cm centered above the floor grid) and a relay to provide acoustical feedback upon correct responses. A computer and associated interface running under MED-PC ver. 2.0 (East Fairfield, VT) recorded the data. A cumulative recorder (Gerbrands Corp., Arlington, MA) was also used to monitor within-session responding. All responses were recorded on the computer for later analysis.

Procedure. Subjects were trained to respond under a repeated acquisition procedure (Winsauer *et al*., 1996). Subjects were trained until the behavior stableized at less than 30% errors. Training normally required 8 to 14 wk to complete. In this procedure the subjects were required to respond on a different key in the presence of each of three different stimuli. The sequence of stimulus presentation remained constant for all sessions (red followed by white followed by amber) and correct positions were chosen from a predetermined list of mixed ordered sequences (sequences included LRC, LCR, CLR, CRL, RLC and RCL). Sequencial presentation of the designated stimulus appeared on all keys. The list was determined such that each sequence was used three times every 18 sessions and no 2 consecutive sessions had the same position designated as correct for the first stimulus. Correct responses resulted in the presentation of the next stimulus in the sequence and incorrect responses resulted in a 2-sec time-out, during which responses had no programed consequences. The same sequence was presented throughout an entire session. Each sequence completion briefly (1 sec) illuminated the feeder light. Correct responses were reinforced with a food pellet after every third completion of the three response sequence (chained second-order fixed-ratio schedule. Thus, a pellet was delivered after every nine correct responses emitted. Each session terminated after the subject received 75 pellets or after 1 hr, whichever occurred first. Nondrugged subjects typically received 75 pellets within 15 min. Nondrugged performance for each subject was usually within 15% of base-line values. Subjects were divided into three groups for testing. Two drugs (and combinations with SR when appropriate) were tested in each group of six rats (in order of testing, group 1: Δ^9-THC and SR; group 2: anandamide and R-methanandamide; group 3:

cannabidiol and morphine). Testing was conducted between 2:00 and 5:00 PM. 6 days/wk.

Drugs. Anandamide and R-methanandamide were obtained from RBI (Natick, MA). Δ^9-THC, morphine sulfate and cannabidiol were provided by NIDA, Research Technical Branch (Rockville, MD). SR was generously provided by Dr. Rinaldi-Carmona and M. Mosse (Sanofi, France). All cannabinoids (anandamide, R-methanandamide, Δ^9-THC, SR and cannabidiol) were prepared as emulsions using alcohol, emulphor, and saline (1:1:18). Anandamide, R-methanandamide and Δ^9-THC were received in solution with ethanol. The drug was isolated by lyophilization, stored in a freezer and prepared no more than 5 days before use. All injections were administered i.p. Anandamide and R-methanandamide were administered 10 min before the session. SR and Δ^9-THC were administered 40 and 30 min before the start of the session, respectively. Morphine sulfate was prepared in saline and administered 15 min before the start of testing. Drug administrations were separated by a minimum of 5 days of either vehicle or noninjection sessions. Administrations of high doses of drugs were separated by at least 2 wk in an effort to minimize the development of tolerance.

Data analysis. The data collected from each session were analyzed in terms of overall response rate (total responses/second, excluding timeouts) and percent errors [(errors)/(errors + corrects) × 100%]. Control values for the rate of responding and percent errors were calculated in each subject by dividing the value of each vehicle session by the average of 10 vehicle sessions in the same subject and multiplying by 100%. Vehicle values plotted represent the mean (±S.E.M.) of control sessions from all subjects. Dose-response data for each subject was divided by the average vehicle value for that subject and expressed as a percent of control. Dose-response values plotted represent the grouped mean (±S.E.M.) of values from all subjects. Within-session cumulative errors by response were plotted as a mean (±S.E.M.) for each dose with six rats in each group. Percent errors and cumulative errors were excluded from the error analysis when the corresponding response rate was less than 5% of the vehicle average.

Statistical analysis. Statistical analysis of data was performed using a Kruskal-Wallis One Way Analysis of Variance on Ranks (single factor, drug treatment) with multiple comparisons (Dunn's method) to isolate groups that differed from vehicle ($P < .05$) using the SigmaStat statistical package (Jandel Scientific, San Rafael, CA). ED_{50} values were estimated using TableCurve 2D (Jandel Scientific), a nonlinear sigmoidal curve-fitting software package employing a Levenburg-Marquardt algorithm (Bevington, 1969). The equation used included four fitting parameters (*response* = A+B/(1+(dose/C) D)). Parameters A and B were modestly constrained to set the minimum effect of the drug near the vehicle values and the maximal effect not to exceed values obtained during administration of the highest dose of the agonist alone.

Results

Effects of cannabinoids. Δ^9-THC produced a significant ($H = 65.5$, $df = 8$, $P < .05$) decrease in the rate of responding and a significant increase ($H = 60$, $df = 8$, $P < .05$) in percent errors at doses of 10 and 18 mg/kg (fig. 1). ED_{50} values for decreasing the rate of responding and increasing percent errors were 4.83 and 10.38 mg/kg Δ^9-THC, respectively (fig. 1, dashed lines). These doses were estimated to decrease the overall response rate to 69% of control and increase percent errors to 205% of control levels. No residual behavioral effects of Δ^9-THC were detected the day after drug administration. Administration of 1 mg/kg SR antagonized the error increasing and rate decreasing effects of Δ^9-THC (3.2–32 mg/kg i.p.) (fig. 1 open symbols). ED_{50} values estimated after the administration of SR were 16.54 mg/kg and 27.13 mg/kg

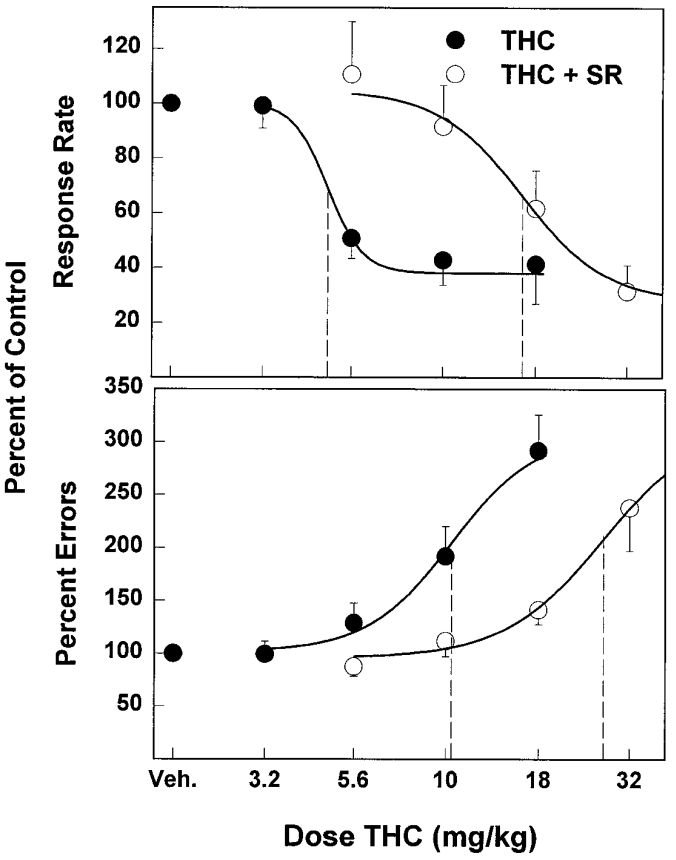

Fig. 1. Effect on rate of responding (top) and percent errors (bottom) as a function of dose of Δ^9-THC alone or in combination with 1 mg/kg SR in a repeated acquisition procedure. Data are expressed as a percentage of vehicle control values. Points left of the dose-effect curves represent the grouped mean (±S.E.M.) of 10 determinations from each of six rats under vehicle conditions. Dashed vertical lines represent estimated ED_{50} (values in text) for decrease in rate of responding and increase in percent errors. Values for each dose represent the grouped mean (±S.E.M.) from six rats determined twice in each rat.

Δ^9-THC on the rate of responding and percent errors, respectively (fig. 1, dashed lines). Based on the increases of ED_{50} estimates, SR produced a 3.4-fold shift to the right in the dose-effect curve for Δ^9-THC on the rate of responding and a 2.6-fold shift to the right in the dose-effect curve for Δ^9-THC on percent errors. For percent errors, the dose-effect curve for Δ^9-THC was shifted and remained parallel to the original curve. Figure 2 (top) shows the effect of Δ^9-THC alone on the within-session distribution of errors. The acquisition process over the first 100 responses is most clearly demonstrated under vehicle conditions (filled circles) by the negatively accelerated nature of the plot. The probability of emitting an incorrect response (*i.e.* the slope of each plot) decreased throughout the first 100 responses, though the decrease was slight at 18 mg/kg Δ^9-THC. Doses above 5.6 mg/kg Δ^9-THC produced a statistically significant ($H = 54$, $df = 4$, $P < .05$) increase in cumulative errors at the 100th response (10 mg/kg Δ^9-THC not plotted). In contrast, the non-centrally active cannabinoid, cannabidiol, had no effect across the range of doses tested (fig. 3).

Effects of anandamide and R-methanandamide. As is shown in figure 4, anandamide (0.01–18 mg/kg i.p.) produced no significant effect on either rate of responding or percent

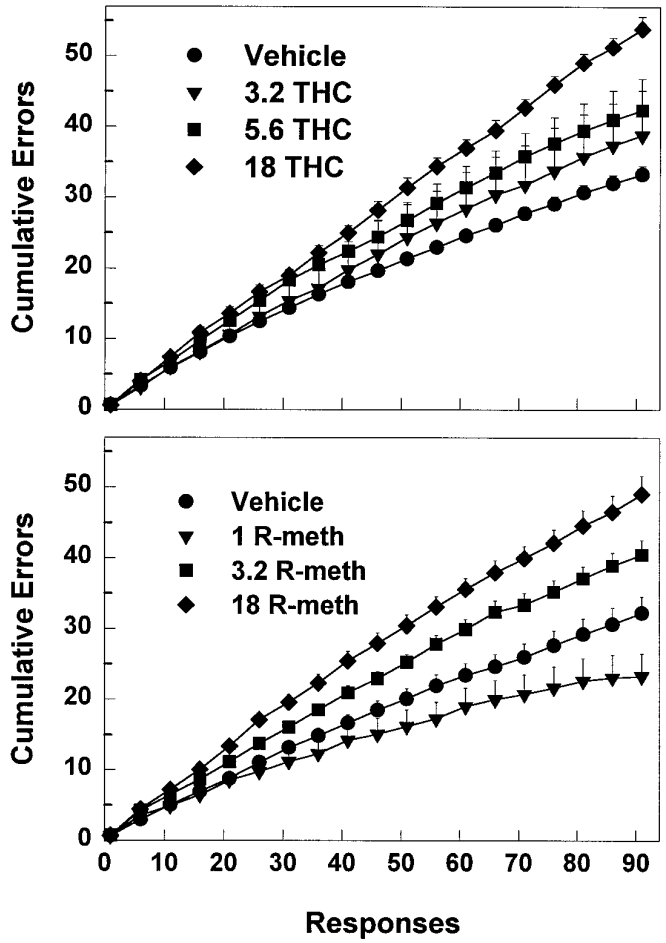

Fig. 2. Effect of Δ^9-THC (top) and R-methanandamide (bottom) on cumulative errors by response for the first 100 responses of the session. Vehicle data represent the mean (±S.E.M.) of 10 determinations and drug data represent the mean (±S.E.M.) of two determinations in each of six rats.

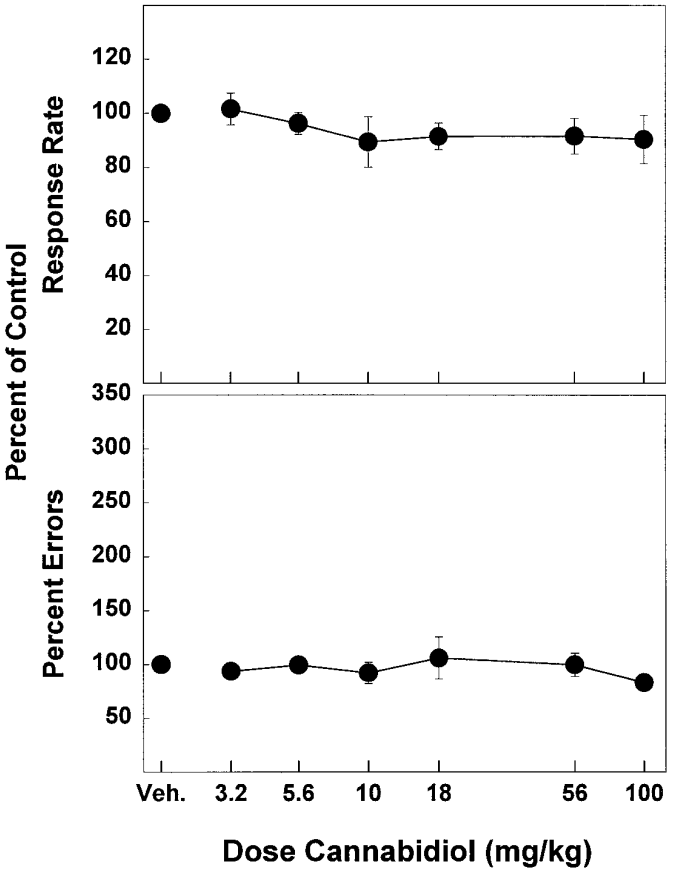

Fig. 3. Effect on rate of responding (top) and percent errors (bottom) as a function of dose of cannabidiol in rats responding under a repeated acquisition procedure. Data are expressed as a percentage of vehicle control values. Points left of the dose-effect curves represent the grouped mean (±S.E.M.) of 10 determinations from each of six rats under vehicle conditions. Values for each dose represent the grouped mean (±S.E.M.) from six rats determined twice in each rat.

errors. The effects of R-methanandamide on the rate of responding and percent errors, alone (closed symbols) and in combination with 1 mg/kg SR (open symbols) are shown in figure 5. R-methanandamide significantly ($H = 64.5$, $df = 9$, $P < .05$) decreased the rate of responding at doses greater than 5.6 mg/kg and increased percent errors at doses above 18 mg/kg ($H = 27.1$, $df = 9$, $P < .05$). The ED_{50} values for decreasing response rate and increasing percent errors were 7.39 and 14.03 mg/kg, respectively. These doses were estimated to decrease the response rate to 56% of control and increase percent errors to 160% of control levels. A dose of 1 mg/kg of SR produced a 1.89-fold shift to the right for rate of responding in the R-methanandamide dose-effect curve and a 3.20-fold shift to the right in the dose-effect curve for percent errors. When 1 mg/kg SR was administered in combination with R-methanandamide the rate of responding was significantly ($H = 64.5$, $df = 9$, $P < .05$) decreased at doses of 10 mg/kg and above and percent errors were significantly increased ($H = 27.1$, $df = 9$, $P < .05$) at a dose of 32 mg/kg R-methanandamide. ED_{50} values for R-methanandamide when administered in combination with SR were 14.03 mg/kg and 30.81 mg/kg for response rate and percent errors, respectively (fig. 5, open circles). Figure 2 (bottom) shows the effect of R-methanandamide (1, 3.2 and 10 mg/kg) on the within-session distribution of errors over the first 100 responses. Note that 1 mg/kg of R-methanandamide produced what might appear to be a slight error reducing effect, but this effect did not reach statistical significance. More than the first 100 responses doses above 3.2 mg/kg R-methanadamide produced a statistically significant ($H = 31.2$, $df = 4$, $P < .05$) increase in errors.

Effects of SR. Administration of SR alone (1–32 mg/kg i.p.) had no significant effect on either rate of responding or percent errors (fig. 6). At the highest dose tested (32 mg/kg) SR produced modest decreases in the rate of responding and increases in percent errors in only a few subjects. Grouped values including unaffected subjects did not reach significance. Except for a few subjects that appeared slightly sedated after the highest dose of SR, informal observation of the animals in their home cages after injection of SR (from 40–6 min. before being placed in the chamber) revealed no effects on motor activity, rearing, sniffing, defecation or aggressiveness.

The effects of morphine. As shown in figure 7, administration of morphine (3.2–10 mg/kg) produced a dose-dependent decrease in the rate of responding and increase in percent errors. Doses above 3.2 mg/kg morphine produced a significant decrease in response rate and increase in percent errors ($H = 36.3$, $df = 6$, $P < .05$; $H = 42.5$, $df = 6$, $P < .05$,

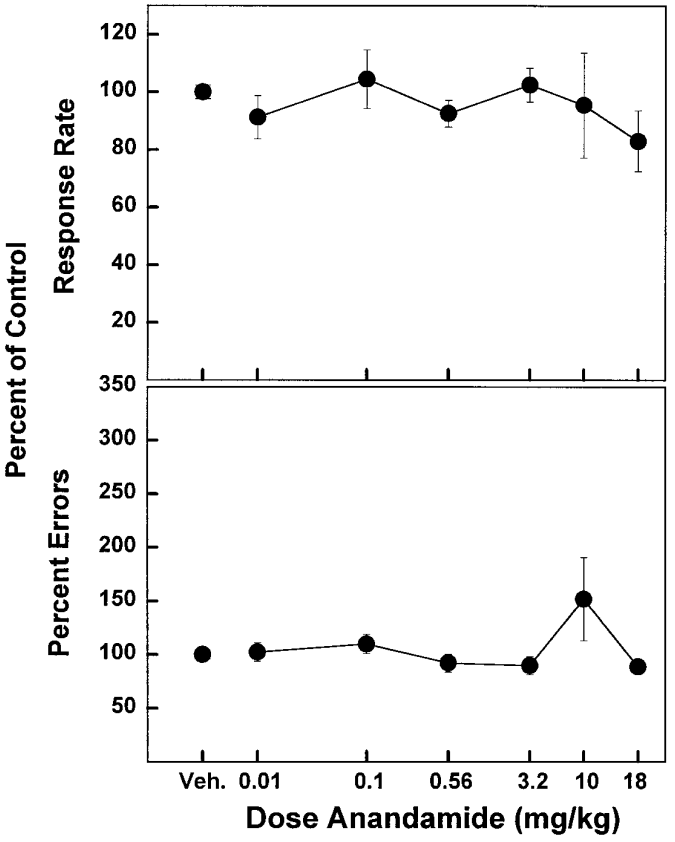

Fig. 4. Effect on rate of responding (top) and percent errors (bottom) as a function of dose of anandamide in a repeated acquisition procedure. Data are expressed as a percentage of vehicle control values. Points left of the dose-effect curves represent the grouped mean (±S.E.M.) of 10 determinations in each of six rats under vehicle conditions. Values for each dose represent the grouped mean (±S.E.M.) from six rats determined twice in each rat.

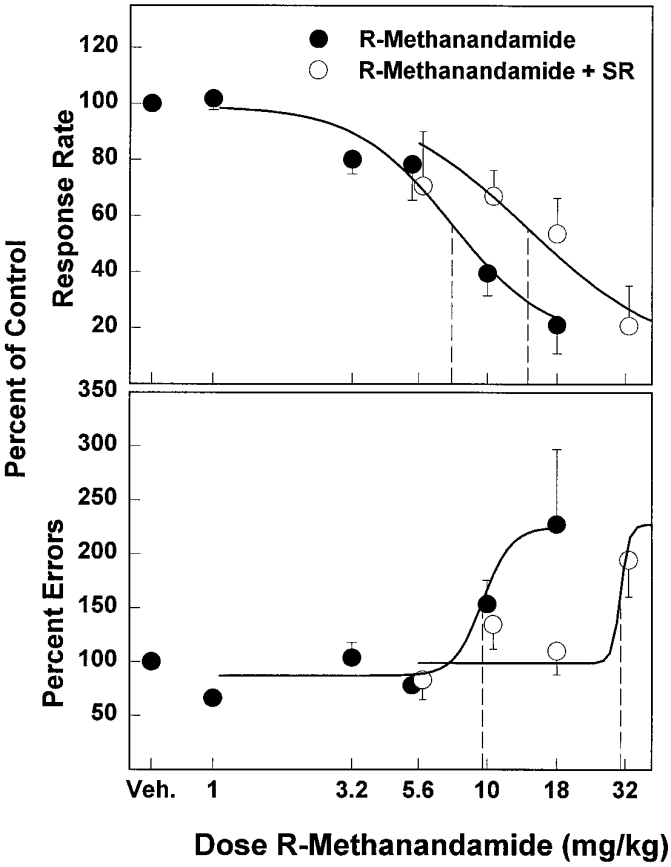

Fig. 5. Effect on rate of responding (top) and percent errors (bottom) as a function of dose of R-methanandamide alone or in combination with 1 mg/kg SR in a repeated acquisition procedure. Data are expressed as a percentage of vehicle control values. Points left of the dose-effect curves represent the grouped mean (±S.E.M.) of 10 determinations in each of six rats under vehicle conditions. Dashed vertical lines represent estimated ED_{50} (values in text) for decreased rate of responding and increased percent errors. Values for each dose represent the grouped mean (±S.E.M.) from six rats determined twice in each rat.

respectively). All rats appeared sedated in a dose-related manner when observed in their home cages before the start of the session. Administration of 1 mg/kg SR did not alter the effects of morphine. SR had no observable interaction with the sedation produced by morphine.

Discussion

Our results suggest that the cannabinoid agonists, Δ^9-THC and R-methanadamide, impair learning as a result of stimulation of the CB1 receptor. The observed effects of Δ^9-THC are consistent with reports that Δ^9-THC disrupts performance of tasks requiring short-term memory (Heyser *et al.*, 1993; Lichtman *et al.*, 1995). Δ^9-THC also decreased response rate. Therefore it might be argued that the observed increase in percent errors was merely the result of the increased time between each response. An increase in the interresponse interval may have increased the difficulty of the task resulting in more errors. Although it is possible that Δ^9-THC only produced a decrease in the rate of responding and the increase in percent errors was therefore merely a consequence of this singular effect, several observations are inconsistent with this explanation. Examination of the effect of Δ^9-THC (fig. 1) shows that the rate of responding was decreased at doses that did not affect percent errors and that increases in percent errors were not accompanied by corresponding decreases in the rate of responding (10 and 18 mg/kg). Lichtman *et al.* (1995) reported that intrahippocampal administration of a cannabinoid agonist produced disruptions in accuracy without affecting completion time in a radial-maze procedure. Another alternative explanation is that our findings are the result of Δ^9-THC's effect on "motivation." However, feeding subjects immediately before testing has been shown to decrease response rate without affecting percent errors (Thompson and Moerschbaecher, 1979). Also, inspection of the chambers after each session revealed that all the subjects consumed all of the food pellets presented and they consistently consumed postsession rations. Finally, it has been demonstrated that other drugs may decrease response rate dramatically without affecting percent errors (Moerschbaecher, *et al.*, 1984). These observations strongly suggest that accuracy and rate of responding are independent measures and support our conclusion that the disruptive effects of Δ^9-THC on accuracy are not simply a result of the decrease in the rate of responding.

The endogenous cannabinoid ligand, anandamide, has previously been shown to produce effects similar to Δ^9-THC on measures of gastric motility and body temperature (Crawley

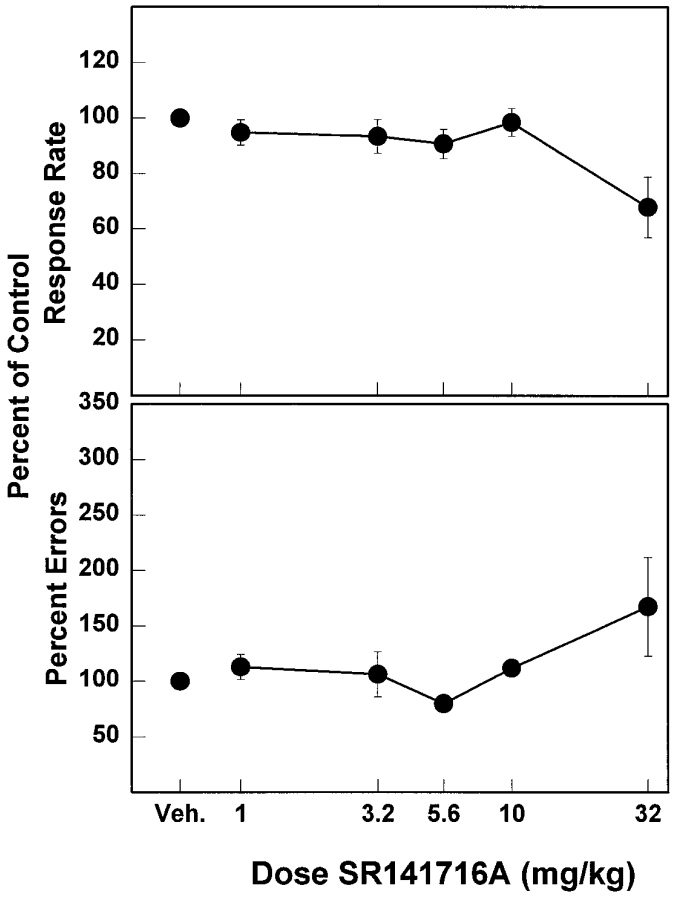

Fig. 6. Effect on rate of responding (top) and percent errors (bottom) as a function of dose of SR in a repeated acquisition procedure. Data are expressed as a percentage of vehicle control values. Points left of the dose-effect curves represent the grouped mean (±S.E.M.) of 10 determinations in each of six rats under vehicle conditions. Values for each dose represent the grouped mean (±S.E.M.) from six rats determined twice in each rat.

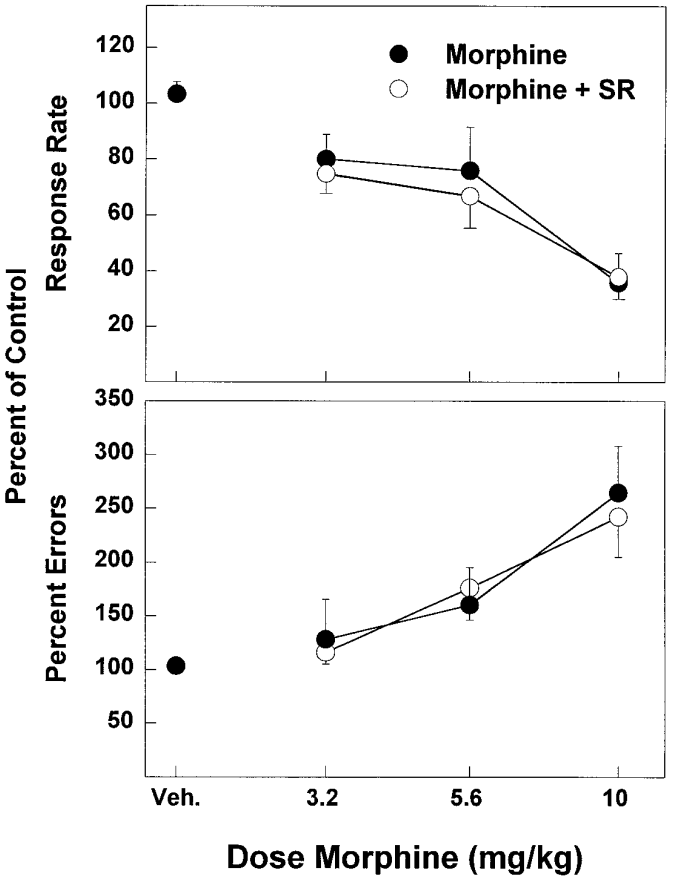

Fig. 7. EEffect on rate of responding (top) and percent errors (bottom) as a function of dose of morphine alone or in combination with 1 mg/kg SR in a repeated acquisition procedure. Data are expressed as a percentage of vehicle control values. Points left of the dose-effect curves represent the grouped mean (±S.E.M.) of six determinations in each of six rats under vehicle conditions. Values for each dose represent the grouped mean (±S.E.M.) from six rats determined once in each rat.

et al., 1993). We found, however, that anandamide had no effect in our procedure. This result is consistent with other reports investigating the amnestic effects of anandamide. Using rats in assays designed to asses memory (the DMTS, radial-maze and delayed nonmatch to position procedures) anandamide alone has no effect (Crawley *et al.*, 1993; Lichtman *et al.*, 1995; Mallet and Beninger, 1996). This lack of an effect may simply reflect a very short duration of action of anandamide in rats. Mallet and Beninger (1996) attempted to address this problem by treating rats with the protease inhibitor PMSF to enhance the activity of anandamide. When PMSF was administered before anandamide, disruptive effects in a delayed non-match to position procedure were observed at doses that did not effect a conditional discrimination task. Despite complications introduced by adding PMSF, this finding suggests that a longer duration of action may be a critical factor for detecting the amnestic properties of anandamides. Our tests using the more metabolically stable (Abadji *et al.*, 1994) analog of anandamide, R-methanandamide, support this suggestion. R-methanandamide was generally equipotent with Δ^9-THC at decreasing the rate of responding and increasing percent errors.

In light of the reported disruptive effects of Δ^9-THC on measures of memory, the discovery of an endogenous cannabinoid agonist has renewed interest in the possibility of manipulating the anandaminergic system to produce nootropic agents. Our results using the cannabinoid antagonist SR alone suggest that the anandaminergic system is not tonically involved in learning in rats responding on a repeated acquisition task. Administration of SR across a wide range of doses produced no effect on either rate or accuracy of responding. It is likely that any improvement in accuracy would have been observable as 1 mg/kg R-methanandamide produced a decrease in percent errors, although this effect did not reach significance (figs. 5 and 2, bottom). The highest dose of SR tested disrupted response rate and percent errors in three subjects. Testing of higher doses of SR may have resolved the ambiguity of this slight trend. The effects obtained with SR alone in our study are consistent with previous reports showing little or no effect of SR when administered alone (Mansbach *et al.*, 1996). The initial characterization by Rinaldi-Carmona *et al.* (1994, 1995) reported that SR produced no effect in assays generally considered very sensitive to the effects of cannabinoid agonists: body temperature, catalepsy and nociception. SR has also been shown to lack effect in pigeons responding under a fixed consecutive number procedure, up to doses as high as 17

mg/kg (Mansbach *et al.*, 1996). The effects of SR reported by Rinaldi-Carmona and the results communicated in this report seem inconsistent with the recent findings of Compton *et al.*, (1996) who reported that administration of SR produced opposite effects to those of Δ^9-THC on locomotor activity in mice. This apparent inconsistency with our findings may be due to either the behavior measured or the species used. Another study using mice has found them to be far more sensitive than rats to the effects of exogenous anandamide (Fride *et al.*, 1995). To our knowledge, however, mice have never been used as subjects in studies using repeated acquisition procedures. Further investigation into the effects of SR on performance in a repeated acquisition task using mice as subjects may help clarify this issue. Although our findings do not support the notion that SR enhances learning in rats, it is possible that SR may exert different effects in other species or procedures.

Strong *in vivo* evidence establishing SR as a cannabinoid antagonist has come from drug discrimination studies. Drug discrimination procedures using animals trained to discriminate Δ^9-THC from saline have reported that SR potently antagonizes the effects of cannabinoid agonists. A dose of 1 mg/kg SR has been shown to produce complete reversal of Δ^9-THC appropriate responding in pigeons (Mansbach *et al.*, 1996), and rats (Perio *et al.*, 1996). In one study up to a 12-fold shift to the right in the Δ^9-THC dose-response curve for rats responding under a drug discrimination procedure was reported (Wiley *et al.*, 1995). In our study, SR shifted the dose-response curves for Δ^9-THC and R-methanandamide approximately one-half log unit to the right. That SR antagonized Δ^9-THC and R-methanandamide but not morphine further extends the actions of SR as a selective cannabinoid antagonist *in vivo*. It should be noted, however, that SR was more effective at antagonizing the rate decreasing effects of Δ^9-THC than those of R-methanandamide. This asymmetry might suggest that some of the rate-decreasing effects of R-methanandamide may be mediated through non-CB1 receptor mechanisms. Interestingly, SR was equally effective at antagonizing the error-increasing effects of R-methanandamide and Δ^9-THC. Together these data further suggest that the disruptive effects of the cannabinoid agonists are selectively mediated through stimulation of the CB1 receptor. Furthermore, the lack of any effect of SR alone suggests that endogenous stimulation of the CB1 receptor may not be involved in learning.

References

Abadji, V., Lin, s., Taha, G., Griffin, G., Stevenson, L. A., Pertwee, R. G. and Makriyannis, A.: (R)-Methanandamide: a chiral novel anandamide possessing higher potency and metabolic stability. J. Med. Chem. **37:** 1889–1893, 1994.

Bevington, P. R.: Data reduction and error analysis for the physical sciences. New York, McGraw Hill, 1969.

Compton, D. R., Aceto, M. D., Lowe, J. and Martin, B. J.: *In Vivo* characterization of a specific cannabinoid receptor antagonist (SR141716A): inhibition of delta-9-tetrahydrocannabinol-induced responses and apparent agonist activity. J. Pharmacol. Exp. Ther. **277:** 586–594, 1996.

Crawley, J. N., Corwin, R. L., Robinson, J. K., Felder, C. C., Devane, W. A. and Axelrod, J.: Anandamide, an endogenous ligand of the cannabinoid receptor, induces hypomotility and hypothermia *in vivo* in rodents. Pharmacol. Biochem. Behav. **46:** 967–972, 1993.

Devane, W. A., Hanus, L., Breuer, A., Pertwee, R. G., Stevenson, L. A., Griffin, G., Gibson, D., Mandelbaum, A., Etinger, A. and Mechoulam, R.: Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science **258:** 1946–1949, 1992.

Fride, E., Barg, J., Levy, D., Saya, E., Heldman, R., Mechoulam, R. and Vogel, Z.: Low doses of anandamides inhibit pharmacological effects of delta-9-tetrahydrocannabinol. J. Pharm. Exp. Ther. **272:** 699–707, 1995.

Herkenham, M., Lynn, A. B., Little, M. D., Johnson, M. R., Melvin, L. S., DeCosta, B. R. and Rice, K. C.: Cannabinoid receptor localization in brain. Proc. Natl. Acad. Sci. **87:** 1932–1936, 1990.

Heyser, C. J., Hampson, R. E. and Deadwyler, S. A.: Effects of delta-9-tetrahydrocannabinol on delayed match to sample performance in rats: Alterations in short-term memory associated with changes in task specific firing of hippocampal cells. J. Pharmacol. Exp. Ther. **264:** 294–307, 1993.

Johnson, L., O'Malley, P. and Bachman, J.: National survey results on drug use from the monitoring the future study, 1975–1993. Rockville, M. D., National Institute on Drug Abuse; 1993.

Lictman, A. H., Dimen, K. R. and Martin, B. R.: Systemic or intrahippocampal cannabinoid administration impairs spatial memory in rats. Psychopharm. **119:** 282–290, 1995.

Mallet, P. E. and Beninger, R. J.: The endogenous cannabinoid receptor agonist anandamide impairs memory in rats. Behav. Pharmacol. **7:** 276–284, 1996.

Mansbach, R. S., Rovetti, C. C., Winston, E. N. and Lowe III, J. A.: Effects of the cannabinoid CB1 receptor antagonist SR141716A on behavior of pigeons and rats. Psychopharm. **124:** 315–322, 1996.

Matsuda, L. A., Lolait, S. J., Brownstein, M. J., Young, A. C. and Bonner, T. I.: Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature **346:** 561–564, 1990.

Miller, L. L. and Branconnier, R. J.: Cannabis: Effects on memory and the cholinergic limbic system. Psychol. Bull. **93:** 441–456, 1983.

Moerschbaecher, J. M. and Thompson, D. M.: Effects of phencyclidine, pentobarbital, and d-amphetamine on the acquisition and performance of conditional discriminations in monkeys. Pharmacol. Biochem. Behav. **13:** 887–894, 1980.

Moerschbaecher, J. M. and Thompson, D. M.: Differential effects of prototype opioid agonists on the acquisition of conditional discriminations in monkeys. J. Pharmacol. Exp. Ther. **226:** 738–748, 1983.

Moerschbaecher, J. M., Mastropaolo, J., Winsauer, P. J. and Thompson, D. M.: Effects of opioids on accuracy of a fixed-ratio discrimination in monkeys and rats. J. Pharmacol. Exp. Ther. **230:** 541–549, 1984.

Perio, A., Rinaldi-Carmona, M., Maruani, J., Barth, F., Le Fur, G. and Soubrie, P.: Central mediation of the cannabinoid cue: activity of a selective antagonist, SR141716A. Behav. Pharmacol. **7:** 65–71, 1996.

Pollard, G. T., McBennett, S. T., Rohrbach, K. W. and Howard, J. L.: Repeated acquisition of three-response chains for food reinforcement in the rat. Drug Dev. Res. **1:** 67–75, 1981.

Rinaldi-Carmona, M., Barth, F., Heaulme, M., Alonso, R., Shire, D., Calandra, B., Congy, C., Soubrie, P., Breliere, J. C. and Le Fur, G.: Biochemical and pharmacological characterization of SR141716A, the first potent and selective brain cannabinoid receptor antagonist. Life Sci. **56:** 1941–1947, 1995.

Rinaldi-Carmona, M., Barth, F., Heaulme, M., Shire, D., Calandra, B., Congy, C., Martinez, J., Neliat, G., Caput, D., Ferrara, P., Soubrie, P., Breliere, J. C. and Le Fur, G.: SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. FEBS Lett. **350:** 240–244, 1994.

Romero, J., Garcia-Palomero, E., Lin, S. Y., Ramos, J. A., Makriyannis, A. and Fernendez-Ruiz, J. J.: Extrapyramidal effects of methanandamide, an analog of anandamide, the endogenous CB1 receptor ligand. Life Sci. **58:** 1249–1257, 1996.

Thompson, D. M. and Moerschbaecher, J. M.: An experimental analysis of the effects of d-amphetamine and cocaine on the acquisition and performance of response chains in monkeys. J. Exp. Anal. Behav., **32:** 3, 433–444, 1979.

Wiley, J. L., Lowe, J. A., Balster, R. L. and Martin, B. R.: Antagonism of the discriminative stimulus effects of delta-9-tetrahydrocannabinol in rats and rhesus monkeys. J. Pharmacol. Exp. Ther. **275:** 1–6, 1995.

Winsauer, P. J., Bixler, M. A. and Mele, P. C.: Comparison of the effects of typical and atypical anxiolytics on learning in monkeys and rats. J. Pharmacol. Exp. Ther. **276:** 1111–1127, 1996.

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