ELSEVIER

Δ^9-Tetrahydrocannabinol Increases Prefrontal Cortical Catecholaminergic Utilization and Impairs Spatial Working Memory in the Rat: Blockade of Dopaminergic Effects with HA966

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The present study examined Δ^9-tetrahydrocannabinol (THC)-induced alterations in monoamine transmission in the rat forebrain as well as the effects of the enantiomers of 3-amino-1-hydroxypyrrolid-2-one (HA966) on the monoamine response to THC. Activation of dopamine (DA) and norepinephrine (NE) but not serotonin (5-HT) turnover in the prefrontal cortex (PFC) was observed after THC (5 mg/kg i.p.) administration. Both enantiomers of HA966 completely prevented the effects of THC on PFC DA turnover and partially blocked the THC-induced rise in NE metabolism. The cognitive consequences of THC exposure were also examined. THC significantly impaired spatial working, but not reference, memory in rats, and this effect was ameliorated by HA966. Thus, HA966 prevents the THC-induced increases in PFC DA turnover and impairments of prefrontal cortical working memory function. Furthermore, these data suggest that cognitive impairments displayed by marijuana self-administering humans may be related to PFC DA hyperactivity and that HA966 may prevent this effect.

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KEY WORDS: *Cannabinoids; Cognition; Delayed-Alteration; Memory; Monoamines; Psychotomimetics*

The mesocortical dopamine (DA) system has been the subject of extensive biochemical and functional research because of its unique responses to stress (Roth et al. 1988; Deutch and Roth 1990) and its involvement in the regulation of working memory (Goldman-Rakic 1987). Mild stress has been shown to selectively increase DA utilization in the medial prefrontal cortex (PFC), the terminal field of the mesocortical DA system (Thierry et al. 1976; for review, see Horger and Roth 1995), and this stress-induced increase in PFC DA turnover is associated with impairments of performance of tasks dependent on working memory function in both rats and monkeys (Murphy et al. 1996a,b).

As with mild stress exposure, administration of such drugs of abuse as amphetamine (During et al. 1992), phencyclidine (Deutch et al. 1987), cocaine (Sorg and Kalivas 1993), opiates (Kim et al. 1986), and Δ^9-tetrahydrocannabinol (THC: Bowers and Hoffman 1986; Bowers and Morton 1994; Chen et al. 1990) increases DA transmission in the PFC. The effects of THC on other monoaminergic systems is less clear. THC does not appear to alter forebrain serotonin (5-HT) activity (Molina-Holgado et al. 1995), whereas the effect of THC on norepinephrine (NE) metabolism in rat brain has yet to be definitively described.

Like mild stress, THC exposure can impair spatial

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working memory performance in rats (Nakamura et al. 1991; Molina-Holgado et al. 1995). This effect has been presumed to be mediated by a disruption of hippocampal function, given the high density of neuronal cannabinoid receptors located in that structure (Herkenham et al. 1991). In contrast, the contribution of increased DA turnover in PFC to the cognitive impairments displayed by THC-treated animals has not been previously studied.

Recent studies in this lab have shown that HA966 (3-amino-1-hydroxypyrrolid-2-one) can modulate the function of the mesoprefrontal DA system. R-(+)HA966, a strychnine-insensitive glycine site partial agonist/functional N-methyl-D-aspartate (NMDA) receptor antagonist, can block increases in PFC but not nucleus accumbens (NAc) DA turnover induced by restraint stress (Morrow et al. 1993), conditioned fear (Goldstein et al. 1994), and the benzodiazepine inverse agonist FG7142 (Murphy et al. 1996b; Horger et al. 1996). Similarly, S-(−)HA966, a γ-hydroxybutyrate-like substance (Singh et al. 1990), prevents increases in PFC DA utilization induced by restraint stress, conditioned fear (Morrow et al. 1995), and FG7142 (Murphy et al. 1996b). Interestingly, S-(−)HA966 appears to be approximately five to 10 times more potent than R-(+)HA966 in blocking hyperdopaminergic states induced by stress.

In the present study, we examined the effects of R-(+)- and S-(−)HA966, agents shown to regulate the mesoprefrontal DA neurons, on THC-induced changes in DA and NE turnover in the PFC, NAc, and striatum (STR) of rats. In addition, we sought to determine whether THC-induced alterations in performance of a spatial delayed alternation task, a measure of spatial working memory shown to be disrupted by increased PFC DA activity (Murphy et al. 1996a), were explored. Finally, the effects of the enantiomers of HA966 on THC-induced alterations in working memory were studied to determine whether these compounds are able to influence the THC-induced cognitive dysfunction.

METHODS

Animals

Male Sprague-Dawley CAMM rats (Charles River Labs, Portage, MI) were used as subjects. The rats were maintained on a 12-h light-dark cycle with the light phase being 7:00 A.M. to 7:00 P.M.

Rats used for cognitive testing were food-restricted for the duration of the experiment to maximize the palatability of food rewards used in the cognitive testing. All animals were fed a limited portion of approximately 5 g of food a day immediately after testing. Water was always available ad libitum. All rats gained approximately 5-10% body weight each week from an initial weight of 150–175 g to a final adult weight of approximately 450–500 g demonstrating that their nutritional requirements were satisfied.

Drugs

(−)-Δ^9-Tetrahydrocannabinol (Research Biochemicals Inc., Natick, MA) was administered i.p. at 5 mg/kg in a volume of 2 ml/kg 30 min before killing or testing. THC (dissolved in ethanol) was dried down under purified nitrogen and then dissolved in a solution of 0.8 ml 25% hydroxy-β-cyclodextrin (RBI, Natick, MA) and 0.2 ml low pH saline (pH = 3.5). Both enantiomers of HA966 were provided through the NIMH Synthesis Program (courtesy of Research Biochemicals Inc, Natick, MA). R-(+)HA966 (15 mg/kg i.p.) and S-(−)HA966 (3 mg/kg i.p.) were delivered in saline at a volume of 1 ml/kg 15 min before THC administration.

Biochemistry

All rats used for biochemical measures were killed during the light phase. The subjects were 225–250 g at death. All rats were habituated to laboratory conditions for 2 days before being killed to reduce the effects of environmental stress. Animals were euthanized by decapitation 30 min after THC. The brains were removed, and the PFC, NAc, and STR dissected on a chilled platform and stored at −70°C.

Tissues were prepared using alumina extraction with dihydroxybenzylamine (DHBA) as an internal standard. Tissues were first sonicated in 400 μl of ice-cold 0.1 mol/L perchloric acid. Then 200 μl of the sonicate was removed for analysis of MHPG by mass spectrometry, while the other 200 μl was then centrifuged for 20 min at 17600*g*. The pellet was retained for analysis of protein content via the method of Lowry et al. (1951). The supernate was brought to pH 11 by addition of 25 μl of 3 mol/L Tris and passed over a column containing acidic alumina (Sigma, St. Louis, MO). The first effluent was discarded. After washing the column with ice-cold water, the column was re-eluted with 150 μl 0.1 mol/L oxalic acid and the eluate saved and analyzed for catecholamines.

Analysis of monoamine levels was conducted with high performance liquid chromotography (HPLC) using electrochemical detection with a glassy carbon electrode at +0.7 V (BAS, West Lafayette, IN) and reversed-phase columns (3-mm C18 beads, 100Å diameter, 10 cm length; Bioanalytical Systems Co., West Lafayette, IN). The mobile phase used was an 8% solution of acetonitrile containing 0.6% tetrahydrofuran, 0.1% diethyl amine, 0.025 mmol/L EDTA, 2.3 mmol/L 1-octane-sulfonic acid, 30 mmol/L sodium citrate, and 13.7 mmol/L sodium dihydrogen phosphate (final pH 3.1). MHPG (free + conjugate) was analyzed in rat brain homoge-

nate via GC/MS according to the methods of Elsworth et al. (1983).

Delayed Alternation Testing

Sixteen rats were tested 5 days a week during the light phase by an experienced tester at the same time every day to preclude circadian effects. The individual rat testers were always blind to any drug treatments.

As noted previously, all rats were food restricted for the duration of the experiment; each rat was fed approximately 5 g of rat chow immediately after testing each day. No rat experienced weight loss during the experiment; in fact, all rats gained 5–10% of their body weight each week until they reached their adult weights of 450–500 g. Miniature chocolate chips were used as a reward during testing.

White noise was broadcast in the testing room during daily experiments to prevent external noises from disturbing performance of the task. The same standard black T-maze was used for both delayed alternation and black/white discrimination testing.

Training and testing were performed according to the methods described previously in Murphy et al. (1996a,b). Rats were trained to perform the task by first habituating them to the testing apparatus for 2 days. During habituation, the rats were allowed to wander freely about the maze. Chocolate chips were noncontingently available in both arms of the maze. Each animal was then trained under force alternation for 2 days. In this paradigm, one of the T-maze arms is physically blocked, forcing the animal to choose the open arm. By alternating open arms from trial to trial, the animal engages in a forced-choice alternation.

After training, the animals began testing on the delayed alternation task. On the first trial, the rat was rewarded for entering either arm. Thereafter, the rats were rewarded for entering the arm not previously chosen for a total of 10 trials per day.

The subjects were kept in the start box of the T-maze between individual trials for the duration of the "delay period." Delays of individual rats were increased progressively for the duration of the experiment to stabilize performance at an 80% criterion. Thus, performance at 90–100% resulted in an increase in the duration of the intertrial delay.

Drug was administered after an animal had performed an average of 75–85% for the 2 previous days. This criterion allowed for detection of both impairment or improvement after drug treatment. Each drug treatment was followed by a 1-week washout period during which the animal received no drug. The drug treatments given in this study were delivered in a randomized Latin Squares design.

Black/White Discrimination Testing

Eight rats were trained to perform a black-white discrimination task in the T-maze to assess the effects of drug treatments on a task that has the same motivational and motor requirements as the delayed alternation but lacks the working memory requirement. In this task, rats traverse the runway and then choose the arm of the appropriate color. The rats are trained to choose either solely the black or white arm. In this way, the rat does not have to "retain" any positional information from trial to trial; it always chooses black or white.

Black and white T-maze arm inserts were used as the positions of the colors varied randomly, and the inserts could easily be adjusted between trials. Each daily session consisted of an equal number of trials with black in the right arm as with white in the right arm.

Training consisted of a 2-day habituation period followed by a 2-day forced choice period as with the delayed alternation. During forced choice, the rats were forced to choose the appropriate color by physically blocking the incorrect arm.

Delays were increased to maintain a 90% criterion of performance. When a subject performed 90% on 2 consecutive days, drug was administered. A 1-week washout period was utilized as with delayed alternation testing.

Statistics

Statistical analysis was performed on a Macintosh IIcx running Statworks (Cricket Software, Philadelphia, PA). Analysis of biochemical data utilized a between-subjects design, whereas in the cognitive testing paradigm, a within-subjects design was used such that each rat's performance on drug was compared with its performance on vehicle. Analysis of variance and t-test were used where appropriate, with a p value $< .05$ considered significant. Data are expressed as mean ± SEM.

RESULTS

Effects of THC on Forebrain Monoamine Turnover

THC (5 mg/kg i.p.) induced a significant increase in PFC DA turnover (DOPAC/DA) 30 min after administration (Figure 1; mean ± SEM for veh = 0.32 ± 0.02, $n = 7$; THC = 0.43 ± 0.03, $n = 7$; $df = 12$; $p = .017$). There were no effects of THC on NAc or STR DA metabolism at the dose studied (Figure 1); however, THC treatment stimulated significant increases in NE turnover (MHPG/NE) in the PFC (Figure 2; veh = 0.19 ± 0.01, $n = 7$; THC = 0.22 ± 0.01, $n = 8$; $df = 13$; $p = .038$) and NAc (Figure 2; veh = 0.02 ± 0.001, $n = 6$; THC = 0.05 ± .01; $n = 5$; $df = 9$; $p = .030$). In contrast, there was no in-

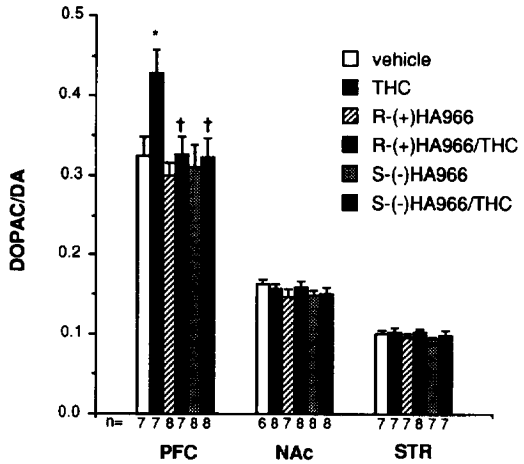

Figure 1. THC significantly increased DA turnover (DOPAC/DA) in the PFC but not NAc or STR of the rat. This effect was blocked by both enantiomers of HA966. All values represent mean ± SEM. *Significantly increased relative to vehicle: $p < .05$. †Significantly reduced relative to THC: $p < .05$.

crease in 5-HT turnover (5-HIAA/5-HT) in the PFC after THC (data not shown).

Blockade of THC's Dopaminergic, but Not Noradrenergic, Effects with HA966

The increased PFC DA turnover after THC was prevented by pretreatment with both enantiomers of HA966: R-(+)HA966 (Figure 1; THC = 0.43 ± 0.03, $n = 7$; R-(+)HA966/THC = 0.32 ± 0.02, $n = 7$; $df = 12$; $p = .019$) or S-(−)HA966 (Figure 1; THC = 0.43 ± 0.03, $n = 7$; S-(−)HA966/THC = 0.32 ± 0.02, $n = 8$; $df = 13$; $p = .014$). It is important to note that, at the doses used presently, neither enantiomer of HA966 significantly altered basal DA metabolism (Figure 1) or levels (Table 1) on its own.

Neither enantiomer of HA966 altered basal NE concentrations in the PFC or NAc. In addition, both enantiomers were unable to significantly reverse THC-stimulated increases in PFC and NAc NE turnover (Figure 2).

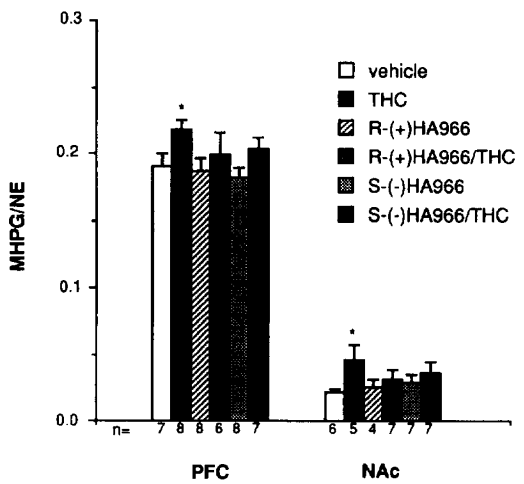

Figure 2. NE turnover was significantly increased in the PFC and NAc after THC administration. Neither enantiomer of HA966 was able to significantly prevent the noradrenergic effects of THC in either area. All values represent mean ± SEM. *Significantly increased relative to vehicle: $p < .05$.

HA966 Prevents THC-induced Impairments of Spatial Working Memory

THC administration 30 min before cognitive testing significantly impaired choice accuracy of rats performing the spatial delayed alternation task (Figure 3; veh = 81.56 ± 2.40% correct; THC = 35.15 ± 4.92% correct; $n = 13$; $df = 12$; $p < .0001$). In contrast, performance of the control, black-white discrimination task was unaltered by THC administration (Figure 3).

The THC-induced cognitive dysfunction was ameliorated by both R-(+)HA966 (Figure 3; THC = 35.15 ± 4.92% correct; R-(+)HA966/THC = 67.50 ± 4.32% correct; $n = 7$; $df = 6$, $p = .022$) and S-(−)HA966 (Figure 3; THC = 35.15 ± 4.92% correct; S-(−)HA966/THC = 59.44 ± 8.35; $n = 7$; $df = 6$; $p = .024$).

DISCUSSION

The present study demonstrates that THC, the psychoactive ingredient in marijuana, has potent activating effects on forebrain catecholaminergic transmission. Specifically, THC administration increases metabolism

Table 1. Effects of THC and HA966 on Brain Dopamine Levels

	Saline	THC (5 mg/kg)
PFC		
Saline	0.40 ± 0.04	0.37 ± 0.03
R−(+)HA966 (15 mg/kg)	0.44 ± 0.03	0.42 ± 0.06
S−(−)HA966 (3 mg/kg)	0.36 ± 0.04	0.38 ± 0.02
NAc		
Saline	48.93 ± 4.78	53.73 ± 3.09
R−(+)HA966 (15 mg/kg)	56.10 ± 3.31	48.84 ± 3.69
S−(−)HA966 (3 mg/kg)	49.95 ± 2.12	55.89 ± 5.14
STR		
Saline	105.69 ± 8.12	103.81 ± 7.33
R−(+)HA966 (15 mg/kg)	97.60 ± 10.48	90.76 ± 6.31
S−(−)HA966 (3 mg/kg)	110.74 ± 7.48	107.95 ± 5.10

Neither THC or HA966, either alone or in combination, significantly alters DA concentrations in the PFC, NAc, or STR of rats, indicating that any observed drug-induced changes in DA turnover are dependent on alterations in DOPAC concentrations. All values represent mean DA concentrations ± SEM.

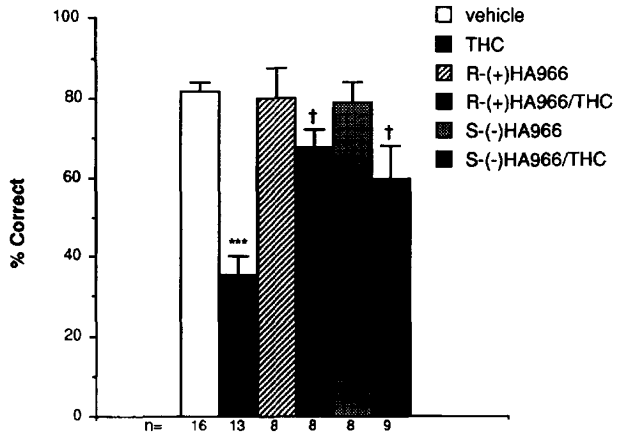

Figure 3. THC significantly impaired choice accuracy of rats performing the delayed alternation. The cognitive dysfunction observed after THC administration is ameliorated by both enantiomers of HA966. Values represent mean ± SEM. ***Significantly decreased relative to vehicle: $p < .001$. †Significantly increased relative to THC: $p < .05$.

of DA in the PFC, but not NAc or STR. In addition, THC is shown here to activate PFC and NAc NE turnover, while having no effects on PFC 5-HT utilization.

The effects of THC on PFC DA turnover have been previously described (Bowers and Hoffman 1986; Bowers and Morton 1994). In addition, THC has been shown to lack effects on NAc DA release in Sprague-Dawley, but not Lewis, rats at low doses comparable to the one used here (Chen et al. 1991). Finally, previous studies have failed to show effects of THC on cortical 5-HT turnover (Molina-Holgado et al. 1995). In contrast, THC's actions on regional NE metabolism have been previously undetailed.

Complementing research from this lab demonstrating that HA966 can selectively regulate the stress-induced activation of the PFC DA system (Morrow et al. 1993; Goldstein et al. 1994; Murphy et al. 1996b), the current data demonstrate that both enantiomers of HA966 can completely block the increases in PFC DA, but not NE, turnover induced by THC. These effects occur at doses of HA966 that are without effects on basal DA or NE turnover, suggesting that HA966 exerts selective modulatory influence on stimulated states of the mesoprefrontal DA neurons. The selectivity of HA966 for the PFC DA system is affirmed by the finding that both enantiomers of HA966 selectively alter phencyclidine-induced increases in PFC DA but not NAc DA or PFC 5-HT turnover (Jentsch et al. 1996).

Recent studies have shown that increased PFC DA turnover induced by a pharmacological stressor impairs spatial working, but not reference, memory and that drugs which prevent the increases in PFC DA turnover after stress, namely HA966, can prevent the stress-induced cognitive dysfunction (Murphy et al. 1996a,b). The present data confirm these findings by showing that THC, which increases PFC DA turnover, impairs performance of a task dependent on spatial working memory and that HA966, which prevents increases in PFC DA turnover induced by THC, ameliorates the impairments in spatial working memory observed after THC administration. These data suggest that increases in PFC catecholamine turnover contribute to the cognitive impairments induced by THC. In addition, it highlights a role for increased PFC DA transmission in this effect and demonstrates that agents which regulate midbrain DA neurons may have important behavioral/cognitive benefits.

Past studies have attributed the cognitive disrupting effects of THC to the high density of cannabinoid receptors localized in the hippocampus (Molina-Holgado et al. 1995); however, the present data show that the mnemonic effects of THC are modulated by activation of the PFC DA system. The mechanism by which THC affects DA systems is unclear; however, cannabinoid receptors appear to be located, at great density, on the striatofugal fibers that inhibit the midbrain DA neurons (Herkenham et al. 1991). THC, an agonist of the neuronal cannabinoid receptor, activates G_i, and thus, inhibits adenylate cyclase and cAMP levels (Howlett et al. 1986). By this mechanism, THC may inhibit the γ-aminobutyric acid (GABA)-containing striatofugal neurons, thereby disinhibiting the ventral midbrain DA neurons. In fact, there is recent electrophysiological evidence that THC weakly activates the DA neurons in the substantia nigra (Miller and Walker 1995). The effects of THC on the more sensitive mesoprefrontal DA neurons may be much greater. We await further electrophysiological studies of the effects of THC on brainstem monoaminergic neurons.

The idea that THC stimulates mesoprefrontal DA neurons is consistent with the observed beneficial effects of HA966. There is a developing body of research describing the effects of HA966 on the firing properties of midbrain DA neurons. Both enantiomers of HA966 have dose-dependent effects on midbrain DA neurons: low doses regularize firing by shifting neurons from burst-firing to tonic-firing modes (McMillen et al. 1992; Shepard et al. 1996), whereas high doses, especially of the S-(−) enantiomer, inhibit impulse flow in DA neurons (Shepard et al. 1993). The burst-firing mode has been associated with increased transmitter release relative to the alternate tonic discharge mode even when firing frequency is controlled for (Gonon 1988); therefore, the regularizing, as well as impulse-flow inhibiting, effects of HA966 have profound implications for DA transmission in the PFC and NAc. Whereas the mechanism of action of HA966 has not been clearly defined, the regularizing effects may be modulated by the NMDA receptor properties of HA966, since a tonic NMDAergic control of firing pattern in midbrain DA

neurons has been demonstrated (Chergui et al. 1993). The high dose inhibitory effects of HA966 are NMDA-independent (Shepard and Lehman 1992) and may be mediated by the $GABA_B$ receptor (Shepard et al. 1996).

In summary, THC increases PFC catecholaminergic turnover in the rat, and HA966 selectively prevents the DA component of that activation. In addition, THC impairs spatial working memory, and this cognitive dysfunction appears to be directly related to the THC-induced hyperdopamingeric state of the PFC. Therefore, the cognitive impairments displayed by marijuana self-administering humans may be related to increased PFC DA transmission and HA966 may be helpful in preventing this effect.

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