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Rapid communication

Physical withdrawal in rats tolerant to Δ^9-tetrahydrocannabinol precipitated by a cannabinoid receptor antagonist

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Abstract

Tolerance to Δ^9-tetrahydrocannabinol (Δ^9-THC) was produced in rats by twice daily injections (15 mg/kg i.p.) for 6.5 days. Administration of the cannabinoid antagonist SR141716A (i.p. or i.c.v.) induced a profound precipitated withdrawal syndrome in Δ^9-THC-tolerant animals. The syndrome was characterized by a disorganized pattern of constantly changing brief sequences of motor behavior. Autonomic signs were not evident. THC-tolerant animals that were treated with vehicle remained quiet throughout the observation period.

Keywords: Marijuana; Withdrawal; Tetrahydrocannabinol

Abrupt discontinuation of heavy use of marijuana results in only mild withdrawal symptoms, if they occur at all (reviewed by Hollister, 1986). Although anecdotal reports of an abstinence syndrome in rats and monkeys have appeared (e.g. Kaymakalan, 1978), quantitative behavioral and physiological studies have revealed at most only mild withdrawal signs (McMillan et al., 1971). These failures to observe profound abstinence signs following discontinuation of chronic use of Δ^9-tetrahydrocannabinol (Δ^9-THC) may result from its long half-life in plasma (Wall et al., 1983), because the slowly waning levels of drug could permit adaptation to occur.

Following chronic heavy intake of opiates, withdrawal symptoms can be precipitated by administration of the competitive opiate antagonist naloxone. This approach to cannabinoid withdrawal was impossible until the development of the competitive cannabinoid receptor antagonist *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide hydrochloride (SR141716A) which was accomplished recently by Rinaldi-Carmona et al. (1994). Here we report that SR141716A precipitates a profound withdrawal syndrome in rats rendered tolerant to Δ^9-THC by repeated injections.

Tolerance was produced in rats ($n = 10$) by injections of 15 mg/kg Δ^9-THC i.p. (National Institute on Drug Abuse, Rockville, MD, USA; suspended in an ethanol : alkamuls-emulphor : saline solution, 1 : 1 : 18) every day between 08.00 h and 10.00 h and again between 16.00 h–18.00 h for 6.5 days. Control animals ($n = 10$) received the vehicle at the same times.

Examination of the hypothermic effects of Δ^9-THC revealed that tolerance had occurred. Administration of Δ^9-THC produced a marked drop in core temperature in 5 animals tested on the first day of this regimen (-0.8 ± 0.05°C), but this effect failed to occur on the third day of treatment with the agonist (-0.06 ± 0.06°C). Furthermore, vocalization and 'popcorn' behavior occurred in 4/5 animals on the first day of the regimen, but these behaviors never appeared in any animal on the third day. These findings are consistent with previous reports of cannabinoid tolerance (McMillan et al., 1971).

To test for precipitated withdrawal, animals were placed in an activity chamber (Digiscan, Columbus Instruments, Columbus, OH, USA) 30 min following the last injection. After 1 h, SR141716A (5 mg/kg, $n = 5$ dissolved in 100% dimethyl sulfoxide) or the

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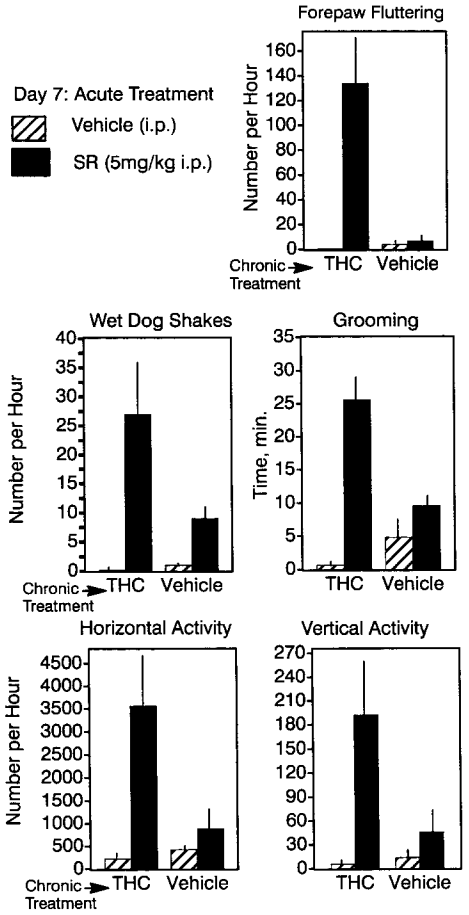

Fig. 1. Rats were either rendered tolerant to Δ^9-THC by twice daily injections for 6.5 days as described in the text, or they served as controls and received the vehicle at the same times. On the test day, half the animals from each of these groups received the selective cannabinoid receptor antagonist SR141716A (5 mg/kg i.p.); the other half received the vehicle. Approximately 10 min following administration of the antagonist, animals that were tolerant to Δ^9-THC displayed a novel behavioral syndrome characterized by hyperactivity and disorganization of behavior, as described in the text. The following types of behavior were observed and tested statistically using analysis of variance (ANOVA) with the Newman-Keuls (NK) post-hoc test for mean differences: bouts of forepaw fluttering, ANOVA: $F(3,15) = 10.1$, $P < 0.001$, NK: $P < 0.05$; wet dog shaking $F(3,15) = 5.8$, $P < 0.01$, NK: $P < 0.05$; time spent grooming, ANOVA: $F(3,15) = 20.7$, $P < 0.0001$, NK: $P < 0.05$); horizontal activity, ANOVA: $F(3,15) = 8.44$, $P < 0.002$, NK: $P < 0.05$; and vertical activity, $F(3,15) = 4.79$, $P < 0.02$, NK: $P < 0.05$. In no case did the antagonist produce a significant effect in non-tolerant rats compared to acute treatment with vehicle.

vehicle ($n = 5$) was administered i.p. to both THC-tolerant and nontolerant rats. Approximately 10 min following administration of the cannabinoid receptor antagonist to Δ^9-THC-tolerant animals, a dramatic abstinence syndrome appeared and lasted throughout the 1 h observation period (Fig. 1).

In nearly constant motion, abstinent rats rapidly alternated between different sequences of behavior, each sequence rarely lasting more than 2 s. Analysis of videotapes revealed sequences such as the following: full turn left, walk backwards two steps, full turn right, raise hindpaw, abort movement – lower hindpaw to floor, wet dog shake, sniff three times, rear, return to horizontal position, half turn to left. Abstinent rats exhibited numerous instances of forepaw fluttering, a tremor-like movement characterized by rapid repetitive medial-lateral movements of the forepaws. This behavior was rarely observed in untreated animals and does not result from any other drug treatment that we are aware of. The significant increases ($P < 0.01$) in horizontal and vertical activity, grooming, wet dog shaking and forepaw fluttering in abstinent rats compared to controls were the result of these unique, rapid and profoundly disorganized patterns of motor activity (Fig. 1). Because the antagonist failed to produce similar effects in nontolerant animals, it would appear that the syndrome we observed was in fact precipitated withdrawal rather than any effect of the antagonist itself.

A second experiment was carried out to determine whether the withdrawal syndrome was mediated by an effect of the antagonist on periventricular structures. In these animals ($n = 48$) cannulae were implanted in the left lateral ventricle and, following recovery, they underwent 6 days of injections as above. Twenty-four hours following the last injection, animals received either SR141716A (100 μg i.c.v) or the vehicle. Withdrawal signs were evident, but the magnitude and complexity of the syndrome was less than that observed following i.p. injection of the antagonist. The most dramatic signs of precipitated abstinence were frequent wet-dog shaking and marked increase in the time spent grooming.

The most striking aspect of the withdrawal syndrome was the rapidly alternating sequences of what appeared to be aborted fragments of organized behavior. This aspect of the syndrome appears to be unique to cannabinoid withdrawal and is not characteristic of the acute effects of any known drug. The site(s) in the brain mediating these effects cannot be stated with certainty. However, it is notable that the highest densities of cannabinoid receptors are found in the basal ganglia (Herkenham et al., 1991), a group of neural circuits whose function may be to organize sequences of behavior (Aldridge et al., 1993; Benecke et al., 1987). Conceivably, the profound disturbance in the sequencing of behavior may have resulted from alterations in the physiology of these circuits.

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