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Dopaminergic system does not play a major role in the precipitated cannabinoid withdrawal syndrome

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KEY WORDS cannabinoids; tetrahydrocannabinol; SR141716A; substance withdrawal syndrome; dopamine D_1 receptors; dopamine D_2 receptors; dopamine antagonists; pruritus; pain

ABSTRACT

AIM: To determine the dopaminergic system involvement in precipitated cannabinoid withdrawal syndrome. **METHODS:** The dopamine D_1 receptor antagonist SCH23390 or the dopamine D_2 receptor antagonist sulpiride was administered to rats chronically treated with either Δ^9-tetrahydrocannabinol (THC) or vehicle. Subjects were then injected with either SR141716A or vehicle and behavior was observed for 1 h. **RESULTS:** Administration of the cannabinoid receptor antagonist SR141716A to animals chronically treated with THC as described by Tsou *et al* (1995) produced a profound withdrawal syndrome. Treatment with dopamine antagonists did not attenuate cannabinoid precipitated withdrawal syndrome in THC tolerant animals while the agonists increased the syndrome. **CONCLUSION:** It is unlikely that the dopaminergic system plays a major role in mediating the behavioral aspects of the cannabinoid withdrawal syndrome.

INTRODUCTION

A cannabinoid withdrawal syndrome was recently described[1,2]. The long life in plasma and lipophilicity of these compounds made it difficult to observe other than mild withdrawal signs in the past due to the likelihood that the brain adjusts to the slowly decreasing levels of the drug[3,4]. The synthesis of the first cannabinoid antagonist[5] made possible the precipitation of a withdrawal syndrome in tolerant animals. Further studies on the cannabinoid withdrawal syndrome quickly followed[6-8], but to date the neural basis of this syndrome is unknown. This study examined the role of the dopaminergic system in mediating the behavioral aspects of the cannabinoid withdrawal syndrome.

MATERIALS AND METHODS

Subjects Sixty-four male Spague-Dawley rats (Charles River Laboratories), approximately 150 - 300 g served as subjects. They were housed in single cages on a 12-h light/dark cycle with food and water *ad lib*.

Drugs All materials used was previously siliconized with Sigmacote (Sigma, St Louis, MO) to prevent the cannabinoid from adhering. THC (NIDA, Rockville, MD) was suspended in ethanol, alkamuls-emulphor, and saline (1 : 1 : 18), and injected at a concentration of 15 mg/kg twice daily. The dopamine D_1 antagonist *R*(+)-SCH23390 (RBI, Natick, MA) was dissolved in saline and injected at a dose of 0.1 mg/kg. The dopamine D_2 antagonist (±)-sulpiride (Sigma) was dissolved in dimethylsulfoxide (DMSO) as was SR141716A (Sanofi Recherche, Montpellier, France) and injected at a dose of 80 mg/kg and 5 mg/kg, respectively.

Injections All injections were given intraperitoneally (ip). Chronic injections consisted of THC or vehicle administered daily between 8 - 10 AM and again between 4 - 6 PM for 6.5 d. On d 7 subjects were injected with THC or vehicle 1 h and 50 min prior to administration of the dopamine antagonist or vehicle.

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Ten minutes later the cannabinoid antagonist or vehicle was administered. Twelve treatment conditions were generated (Tab 1).

Testing An hour after the last chronic injection each animal was placed for 1 h of habituation in a Digiscan Activity monitor (Columbus Instruments, Columbus, OH). After the acute administration of a dopamine antagonist together with SR141716A or vehicle each animal behavior was recorded for 1 h. The horizontal and vertical activity was measured by the activity monitor as the number of photobeams broken between the photocells on the walls of the apparatus. The number of wet dog shakes, scratches with hindpaw, mouth movements, forepaw flutters were also recorded as was the amount of time in minutes that each subject spent grooming.

Data analysis A three way ANOVA was used between chronic treatment (vehicle or THC), acute treatment (vehicle or SR), and dopamine effect (D_1 antagonism, D_2 antagonism or vehicle). The Bonferroni t-test was used for post hoc comparisons of means.

RESULTS

General activity measures significantly increased by the withdrawal syndrome include horizontal activity ($F_{1,52} = 64$, $P < 0.01$, Fig 1A), vertical activity ($F_{1,52} = 21$, $P < 0.01$, Fig 1B). Mouth movements in this study were also increased by the withdrawal ($F_{1,52} = 14$, $P < 0.01$, Fig 1C). Specific withdrawal measures such as forepaw flutters ($F_{1,52} = 29$, $P < 0.01$, Fig 2A) and wet dog shakes ($F_{1,52} = 21$, $P < 0.01$, Fig 2B) were also increased.

Increased grooming ($F_{1,52} = 47$, $P < 0.01$, Fig 3A) and scratching ($F_{1,52} = 35$, $P < 0.01$, Fig 3B) were found in this study to be associated to the administration of the cannabinoid antagonist both in chronic vehicle or THC tolerant animals.

The doses of SCH23390 or sulpiride employed slightly decreased activity in intact animals in general but only in the case of horizontal activity and D_1 antagonist was this decrease significant ($P < 0.01$, Fig 1A). None of the dopamine antagonists significantly attenuated the withdrawal syndrome. Only vertical activity ratings were significantly affected by dopamine antagonist treatment ($F_{2,52} = 4$, $P < 0.05$, Fig 1B) and grooming ($F_{2,52} = 7$, $P < 0.01$, Fig 3A) was attenuated by the dopamine D_2 antagonist

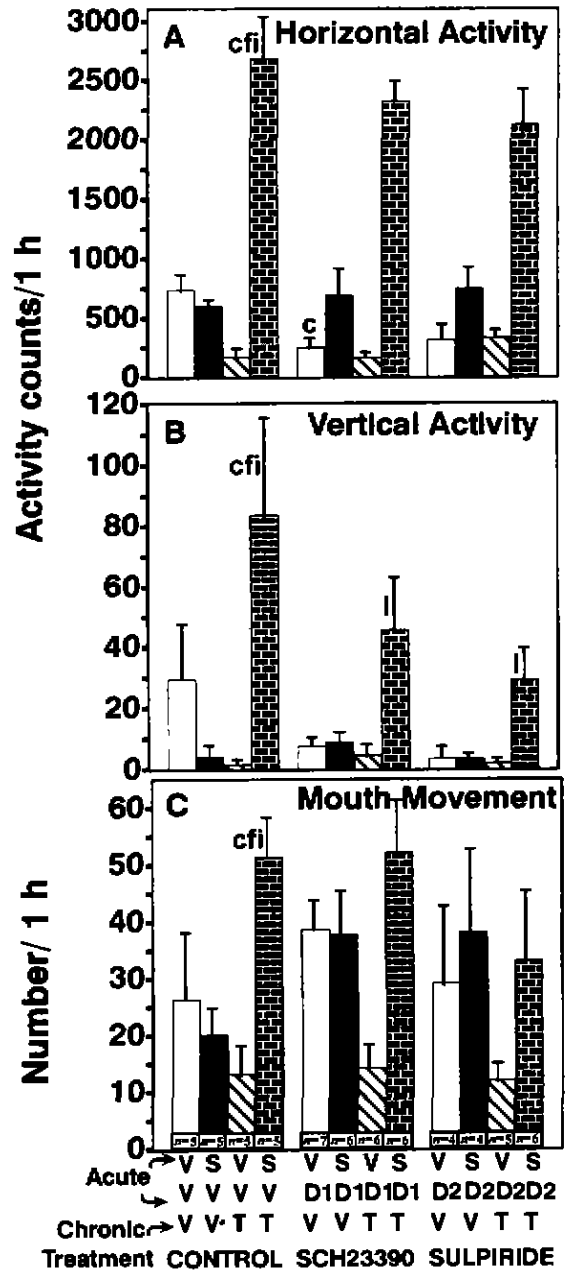

Fig 1. **A: The number of beam breaks along an horizontal plane during the hour long observation period. B: The number of beam breaks along a vertical plane during the hour long observation period. C: The number of mouth movements per hour for all treatment conditions.** $^cP < 0.01$, $^fP < 0.01$, $^iP < 0.01$ **respectively** *vs* **the rest of control groups (VVV, VVS, TVV).** $^1P < 0.01$ *vs* **the control precipitated withdrawal group (TVS).** $\bar{x} \pm$ SEM. **(V = vehicle; T = Δ^9-THC; D_1 = dopamine D_1 antagonist; D_2 = dopamine D_2 antagonist; S = SR141716A).**

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Tab 1. Schematic of the experimental groups employed in this study.
*chronic (6.5 d) #before test; SR = SR141716A SCH = SCH23390 SUL = SULPIRIDE VEH = VEHICLE.

CONTROL-WITHDRAWAL GROUPS				D_1 ANTAGONIST GROUPS				D_2 ANTAGONIST GROUPS			
THC*	THC*	VEH*	VEH*	THC*	THC*	VEH*	VEH*	THC*	THC*	VEH*	VEH*
VEH#	VEH#	VEH#	VEH#	SCH#	SCH#	SCH#	SCH#	SUL#	SUL#	SUL#	SUL#
SR#	VEH#	SR#	VEH#	SR#	VEH#	SR#	VEH#	SR#	VEH#	SR#	VEH#

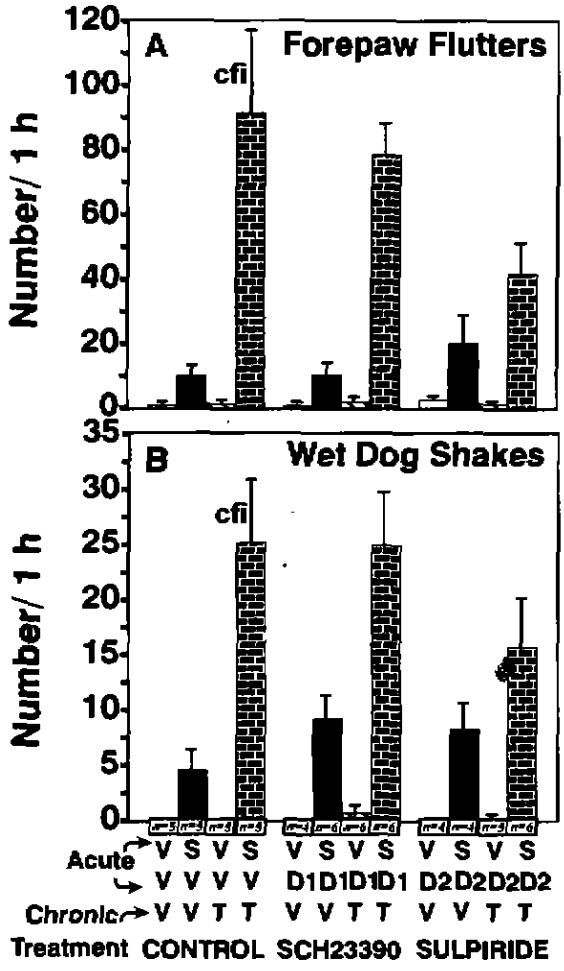

Fig 2. A: The number of forepaw flutters per hour for all treatment conditions. B: The number of wet dog shakes per hour for all treatment conditions. $^cP<0.01$, $^fP<0.01$, $^lP<0.01$, respectively, *vs* the rest of control groups (VVV, VVS, TVV). $\bar{x} \pm$ SEM. (V = vehicle; T = Δ^9-THC; D_1 = dopamine D_1 antagonist; D_2 = dopamine D_2 antagonist; S = SR141716A).

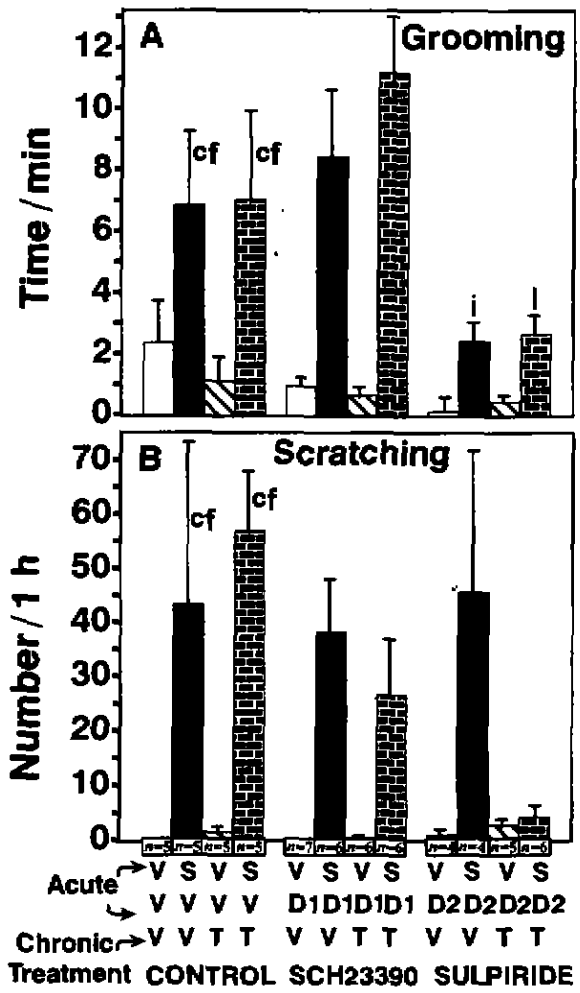

Fig 3. A: The number of minutes spent grooming during the hour long observation period. B: The number of scratches with hindpaw during the hour long observation period. $^cP<0.01$, $^fP<0.01$ respectively *vs* the vehicle control groups that did not receive the cannabinoid antagonist (VVV, TVV). $^lP<0.01$, $^lP<0.01$ *vs* their respective control groups without dopamine D_2 antagonist (VDV, TDV). $\bar{x} \pm$ SEM. (V = vehicle; T = Δ^9-THC; D_1 = dopamine D_1 antagonist; D_2 = dopamine D_2 antagonist; S = SR141716A).

in both groups treated with SR141817A ($P<0.01$).

Administration of the dopamine D_1 agonist SKF82958 or the dopamine D_2 agonist quinpirole to

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withdrawal animals increased the withdrawal syndrome and induced seizures (data not shown).

DISCUSSION

The precipitated cannabinoid withdrawal syndrome described by Tsou *et al* (1995) was reproduced in this study. Two additional behavioral measures, mouth movements and scratching, were also observed to be increased during precipitated withdrawal.

Neither a dopamine D_1 nor a dopamine D_2 antagonist attenuated the withdrawal syndrome which rules out a major role of the dopaminergic system in mediating the hyperactivity observed in cannabinoid withdrawal. A recent work reported a decrease in the activity of the mesolimbic dopaminergic system during cannabinoid withdrawal as well as in opioid withdrawal[7,9].

An independent effect of the cannabinoid antagonist on grooming and scratching behavior was observed. Itching has been related as a subthreshold pain stimulus[10]. Accordingly, cannabinoid antagonism has recently proven to decrease pain thresholds[11]. Itching is induced by dopamine D_2 agonists[12] and this may mediate the increases in itching produced by the cannabinoid antagonism since it was attenuated by the dopamine D_2 antagonist.

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多巴胺受体系统在诱发产生的大麻酚类戒断症状中不起主要作用

R971.2

关键词 大麻酚类; 四氢大麻酚; SR141716A; 物质禁断综合症; 多巴胺 D_1 受体; 多巴胺 D_2 受体; 多巴胺拮抗剂; 瘙痒症; 疼痛

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