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RAPID COMMUNICATION

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A behavioural model to reveal place preference to Δ9-tetrahydrocannabinol in mice

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Abstract *Rationale:* The rewarding properties of Δ9-tetrahydrocannabinol (THC) are difficult to demonstrate in rodents using standard procedures. *Objective:* To evaluate the motivational responses of THC in the place conditioning paradigm in mice after minimizing the dysphoric effects of the first drug exposure and/or the consequences of its pharmacokinetic properties. *Methods:* Mice were conditioned to THC (1 or 5 mg/kg) using an unbiased procedure with an elevated number of pairings and long conditioning time. *Results:* A place aversion was observed with 5 mg/kg THC using a standard protocol. Similar results were obtained when the CB-1 receptor antagonist SR 141716A (1 mg/kg) was administered immediately after each THC conditioning period. However, mice receiving a priming THC injection and conditioned 24 h later showed a place preference with 1 mg/kg THC and no effect with 5 mg/kg THC. *Conclusion:* THC produces a clear place preference in mice by using a long period of conditioning and avoiding the possible dysphoric consequences of the first drug exposure.

Key words Δ9-Tetrahydrocannabinol · Mouse · Reward · Place preference · SR 141716A

Introduction

Δ9-tetrahydrocannabinol (THC) is the psychoactive constituent of *Cannabis sativa*, the most widely consumed illicit drug (Adams and Martin 1996). However, the rewarding properties of THC in the currently used rodent models are controversial. Thus, cannabinoid agonists fail to induce self-administration behaviour in rats (Leite and Carlini 1974; Van Ree et al. 1978), but some agonists of the CB-1 receptors (Devane et al. 1988), such as WIN 55212-2 (D'Ambra et al. 1992), can be self-administered in mice (Martellota et al. 1998). In the place preference paradigm, cannabinoid administration has been shown to produce place aversion in rats (Parker and Gillies 1995; McGregor et al. 1996; Sañudo-Peña et al. 1997; Mallet and Beninger 1998) and mice (Hutcheson et al. 1998), although one early study has reported the occurrence of place preference in rats under particular experimental conditions (Lepore et al. 1995). Various possibilities have been proposed to explain the failure to find a clear rewarding effect of THC in these models. One possibility is the pharmacokinetic profile of the drug, which has a relatively long half-life (McGregor et al. 1996). Another possible explanation is that the first exposure to cannabinoids would produce important dysphoric actions which mask the rewarding effects of the drug (Parker and Gillies 1995; McGregor et al. 1996). The purpose of this study was to test the possibility of revealing rewarding effects of THC in the place preference paradigm in mice by minimizing the dysphoric effects and the consequences of its pharmacokinetic properties.

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Materials and methods

Male CD1 mice (Charles River, France) weighing 22–24 g were housed ten per cage and acclimatized to the laboratory conditions (12 h light: 12 h dark cycle, 21±1°C room temperature) 1 week before the experiment. Food and water were available ad libitum. Behavioural tests and animal care were conducted in accordance with the standard ethical guidelines (NIH, publication no. 85-23, revised 1985) and approved by the local ethical committee. THC (Sigma, UK) was dissolved in a solution of 5% ethanol, 5% cremophor El and 90% distilled water, and injected in a volume of 0.1 ml per 10 g body weight. The CB-1 receptor antagonist SR 141716A (Sanofi Recherche, France) (Rinaldi-Carmona et al. 1994) was dissolved in a solution of 10% ethanol, 10% cremophor El and 80% distilled water, and injected in a volume of 0.2 ml per 10 g body weight. An unbiased place conditioning procedure was used to evaluate the rewarding properties of THC. The apparatus

Table 1 Effects induced by THC in the place conditioning paradigm in mice

Experiment	Dose of THC	*n*	Pre-conditioning time		Post-conditioning time			Scores
			Drug-paired compartment	Vehicle-paired compartment	Drug-paired compartment	*df* and *t* values	Vehicle-paired compartment	
	Vehicle	10	596±31	604±31	521±21	*t*(1.9)=1.31	679±52	–74±66
(A)	1.0 mg/kg	9	635±36	565±36	653±75	*t*(1.8)=0.21	547±75	18±87
	5.0 mg/kg	8	594±48	606±48	449±65*	*t*(1.7)=2.64	751±65	–144±85
(B)	1.0 mg/kg	8	597±68	603±68	531±89	*t*(1.7)=1.28	689±89	–66±52
	5.0 mg/kg	8	607±26	593±26	386±80**	*t*(1.7)=3.59	814±80	–221±62
	Vehicle	14	597±25	603±25	527±38	*t*(1.13)=1.45	673±38	–69±48
(C)	1.0 mg/kg	14	588±26	798±47	612±26***	*t*(1.13)=–4.42	402±47	210±48[a]
	5.0 mg/kg	9	623±46	577±46	574±90	*t*(1.8)=0.52	626±90	–49±94
(D)	1.0 mg/kg	13	607±27	593±27	798±45***	*t*(1.12)=–4.56	402±45	191±42[a]
	5.0 mg/kg	9	613±26	587±26	513±105	*t*(1.8)=1.06	687±105	–100±94

Values represent time in seconds spent in each compartment during the pre-test and test day measurements (means±SEM). Score values are the difference between the post-conditioning and pre-conditioning time spent in the drug-paired compartment. (A) First experiment, (B) second experiment, (C) third experiment, (D) fourth experiment. *P<0.05, **P<0.01, ***P<0.001 versus time in drug-paired compartment on the pre-conditioning day (two-tailed student's paired *t*-test). [a]P<0.01 versus vehicle group (Dunnett's *t*-test). *df* degree of freedom

consisted of two main square conditioning compartments (15×15×15 cm) separated by a triangular central division (Maldonado et al. 1997). The light intensity within the conditioning chambers was 30±5 Lux. The movement and location of the mice were monitored by computerized monitoring software (Videotrack, View Point, Lyon, France) with images relayed from a camera placed above the apparatus. During the preconditioning phase, drug naive mice were placed in the middle of the central division and had free access to both compartments of the conditioning apparatus for 20 min, with the time spent in each compartment recorded. For the conditioning phase, an elevated number of pairings (five pairings with THC and five pairings with vehicle) and a long conditioning time (45 min) were used in all the assays. The drug-assigned compartment could be either the most or the least preferred. Care was taken to ensure that treatments were counterbalanced as closely as possible between compartments. Control animals received vehicle every day. The test phase was conducted exactly as in the preconditioning phase, i.e. free access to each compartment for 20 min. Mice were exposed only once to the preconditioning and test phases. In the first experiment, mice were injected with vehicle or THC (1 and 5 mg/kg, IP) and then immediately confined in the conditioned compartment. In a second experiment, we investigated if the long duration of THC effects could reduce the association between drug and environmental stimulus. For this purpose, the cannabinoid receptor antagonist SR 141716A (1 mg/kg, IP) was administered immediately after each 45-min conditioning period, before animals were returned to the home cage. Finally, to exclude the eventual dysphoric effects induced by the first THC administration, we performed another set of experiments (third and fourth experiment), in which mice received a single THC injection in the home cage 24 h before starting the place preference conditioning procedures. Values for time spent by each animal in drug-paired compartment during the pre-conditioning and post-conditioning measurements were compared by using a two-tailed Student's paired *t*-test. A score was calculated for each mouse as the difference between the post-conditioning and pre-conditioning time spent in the drug-paired compartment. Score values were compared by using one-way ANOVA (between subjects) followed by post hoc comparisons using Dunnett's *t*-test.

Results

No significant effects were revealed when comparing score values by one-way ANOVA between subjects in the first experiment [F(2,26)=1.23, NS]. However, within-group comparisons for time spent in drug-paired side during the pre-test and test days revealed a significant place aversion with 5 mg/kg THC (Table 1). In the second experiment, the administration of the CB-1 antagonist SR 141716A (1 mg/kg, IP) after each drug pairing failed to reveal any significant change on the score values [F(2,25)=1.92, NS], and as in the previous assay, a significant place aversion was revealed by using within-group comparisons with 5 mg/kg THC. Interestingly, a priming THC exposure 24 h before the first conditioning day induced significant differences in the score values of the third experiment [F(2,36)=7.46, P<0.01]. Thus, post-hoc analysis revealed a significant place preference with 1 mg/kg THC, whereas no effects were observed with 5 mg/kg. In agreement, within-group comparisons revealed in this experiment a significant increase of the time spent in the THC-paired compartment on the test day only in mice receiving 1 mg/kg THC. The same behavioural responses were observed in the fourth experiment, when animals received a priming THC exposure 24 h before conditioning and were injected with SR 141716A (1 mg/kg, IP) after each THC conditioning pairing [F(2,35)=7.20, P<0.01].

Discussion

The present results indicate that THC is able to induce rewarding and aversive effects in the place conditioning paradigm in mice, depending on the dose and the experimental design used. Indeed, a place aversion was obtained with 5 mg/kg THC, while no motivational responses were induced with 1 mg/kg when using long periods of conditioning and an elevated number of pairings. In contrast, a clear place preference was obtained with 1 mg/kg THC when mice received a previous priming THC exposure. These data suggest that one of the major factors affecting the instatement of the rewarding properties of THC in the place conditioning paradigm could be the consequences of the possible dysphoric effects induced by the first exposure to the drug.

Further evidence for this hypothesis is provided by the lack of place aversion with a higher dose of THC (5 mg/kg) when avoiding the conditioning effects of the first exposure. Interestingly, acute injections of different cannabinoid agonists induce both anxiolytic- and anxiogenic-like behavioural reactions in rodents. Indeed, first exposure to a large range of doses of the cannabinoid agonist HU-210 produces an anxiety-like response in the defensive withdrawal test in rats (Rodriguez de Fonseca et al. 1997), and an increased aversion to the open arms of the elevated plus maze has been reported after acute injection of THC (10 and 20 mg/kg) in rodents (Onaivi et al. 1995). These anxiogenic-like effects could be mediated by a release of hypothalamic corticotropin-releasing factor (Eldridge and Landfield 1992; Rodriguez De Fonseca et al. 1997). In contrast, the synthetic cannabinoid nabilone (0.01 and 0.1 mg/kg) produced opposite effects to those of THC in the elevated plus maze, i.e. increased the time spent in the open arms (Onaivi et al. 1995). These results are in agreement with the opposite motivational responses induced by THC in the place preference paradigm depending to the dose and the context.

Given after each THC pairing, the specific cannabinoid receptor antagonist SR 141716A failed to reveal or to modify any THC-induced conditioned response supporting that the long duration of cannabinoid effects was not relevant in this paradigm. However, the relatively long pairing time (45 min) used in our experiments could minimize the possible consequences of the pharmacokinetic properties of THC. Therefore, it cannot be excluded that these properties could be relevant for the lack of motivational responses of THC when using other protocols of place preference (Parker and Gillies 1995; McGregor et al. 1996; Hutcheson et al. 1998) or self-administration techniques. Nevertheless, THC self-administration in animals has never been demonstrated, although the cannabinoid receptor agonist WIN 55,212-2 has been reported to be self-administered in mice under appropriate conditions (Martellota et al. 1998).

Previous biochemical studies have shown that THC, as well as other drugs of abuse (Di Chiara et al. 1991), produces neurochemical changes on the dopaminergic mesolimbic system (French et al. 1997; Tanda et al. 1997; Gessa et al. 1998) that could underlie a possible rewarding effect. The present results provide a model that clearly demonstrates the existence of such rewarding properties of THC in mice. This behavioural model of THC-induced conditioned place preference can be useful to elucidate the neurobiochemical mechanisms involved in cannabinoid rewarding effects.

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