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RAPID COMMUNICATION

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Selective inhibition of sucrose and ethanol intake by SR 141716, an antagonist of central cannabinoid (CB1) receptors

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Abstract SR 141716, a selective central CB1 cannabinoid receptor antagonist, markedly and selectively reduces sucrose feeding and drinking as well as neuropeptide Y-induced sucrose drinking in rats. SR 141716 also decreases ethanol consumption in C57BL/6 mice. In contrast, blockade of CB1 receptors only marginally affects regular chow intake or water drinking. The active doses of SR 141716 (0.3–3 mg/kg) are in the range known to antagonize the characteristic effects induced by cannabinoid receptor agonists. These results suggest for the first time that endogenous cannabinoid systems may modulate the appetitive value of sucrose and ethanol, perhaps by affecting the activity of brain reward systems.

Key words Sucrose intake · Ethanol consumption · Cannabinoid receptor · SR 141716 · Rats · Mice

Introduction

Marijuana (*Cannabis sativa*) is one of the oldest and most widely used drugs in the world. The major psychoactive ingredient of the marijuana plant is Δ^9-tetrahydrocannabinol (Δ^9-THC). By stimulating cannabinoid (CB1) G-protein coupled receptors (Herkenham et al. 1990), natural and synthetic cannabinoid agonists produce their characteristic motor, cognitive and analgesic effects. Cannabinoids have also been reported to possess appetite-stimulating properties in humans (Mattes et al. 1994) and to enhance or exacerbate sucrose consumption in rodents, even when inducing overall anorexia (Sofia and Knoblock 1976; Brown et al. 1977).

Recently, Rinaldi-Carmona et al. (1994) reported that SR 141716 [*N*-piperidino-5-(4-chlorophenyl)-1-(2, 4-dichlorophenyl)-4-methylpyrazole-3-carboxamide] bound with high affinity to central (CB1) but not peripheral (CB2) cannabinoid receptors and acted as an antagonist against a variety of pharmacological and behavioural cannabinoid effects in rodents. The availability of such a tool, together with the possible existence of an endogenous cannabinoid ligand (anandamide) (Devane et al. 1992), prompted us to study the effect of SR 141716 on appetitive behaviour including spontaneous or neuropeptide Y (NPY)-induced sucrose intake in rats, and ethanol consumption in C57BL/6 mice.

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Materials and methods

Standard laboratory conditions

Animals were housed under standard conditions [normal 12-h light-dark cycle (rats) or a reversed light schedule (mice); constant room temperature 21°C] with food and water ad libitum except where otherwise noted. All procedures were approved by the Comité d'Expérimentation Animale (Ethical Committee) of Sanofi Recherche.

Spontaneous sucrose feeding

One week prior to the beginning of the experiment, male Wistar rats (210–220 g; CERJ, France) housed eight per cage were placed on a food restriction schedule (13 g standard chow/day per rat). One group were given a small quantity of sucrose pellets (45 mg, Campden) in their home cage to familiarize them with this food. Animals (n = 12 per group) were individually tested in an open-field (76 × 76 × 50 cm) for 30 min daily (4 days) sessions, where they had free access to sucrose pellets and regular rat chow or only regular

rat chow. The amount eaten by each rat was measured by weighing separately sucrose pellets and chow before and after each session. Animals were given SR 141716 or vehicle intraperitoneally (IP, 0.5 ml/100 g) 30 min before each session.

Spontaneous sucrose drinking

One week prior to testing, male Sprague-Dawley rats (190–210 g; Charles River, France) caged individually were given access to a sucrose solution (5%) in their home-cage during daily 4 h sessions (without food and water). On the day of the test, the amount drunk by each rat (n = 6–8 per group) was measured by weighing each bottle at the start and every hour during a 4-h drinking session. SR 141716 or vehicle was given orally (PO, 0.2 ml/100 g) 60 min before the drinking session. As a control, the effect of SR 141716 (PO, 60 min before test) was also studied on water intake (4-h session) after overnight water deprivation.

NPY-induced sucrose drinking

The injection of NPY (2.5 µg/5 µl) or vehicle was made free-hand into the lateral ventricle (ICV) of non-restrained male Sprague-Dawley rats (120–140 g ; Charles River, France) by means of a 50 µl microsyringe and a 10 mm calibrated needle (final length below the skin: 3.5 mm). The experimental conditions were as described above, except that the animals (n = 10–15 per group) were not familiarized with the 5% sucrose solution. SR 141716 or vehicle was administered (PO) 60 min before NPY.

Ethanol consumption

One week before testing, 8-week-old male C57BL/6 mice (Iffa Credo, France) were singly housed in cages equipped with two bottles of tap water. Then, mice (n = 10–20 per group) were subjected to four daily 6-h test sessions in their home cage, during which they were given free access to one bottle of water and one filled with a 10% v/v ethanol solution. The quantities of water and ethanol consumed were measured by weighing the bottles before and after the sessions. SR 141716 or vehicle was injected by the subcutaneous route (SC, 0.4 ml/20 g) 30 min before each session.

Drugs

SR 141716 (Sanofi Recherche, Montpellier, France) was suspended with 0.1% Tween 80 in distilled water. NPY(1–36) (Neosystem) was solubilized in 0.9% NaCl with 1% bovine serum albumin.

Results and discussion

The results obtained in food-restricted rats show that SR 141716 dose-dependently reduced spontaneous sucrose eating at doses which did not modify regular food intake in the no choice situation (Table 1). Furthermore, upon repeated administration of the highest dose, tolerance developed towards the reduction of regular food intake but not for suppression of sucrose ingestion [during the four daily sessions, quantities (g) of regular chow eaten by control rats were: 3.4 ± 0.3; 3.3 ± 0.3; 2.8 ± 0.2; 2.9 ± 0.2 while for rats

Table 1 Effects of SR 141716 on sucrose intake in rats and ethanol consumption in mice. Data are the mean ± SEM quantities of sucrose, regular chow, water or ethanol ingested, expressed in grams. Sucrose pellets and control regular chow eaten were measured over a 30-min period during 4 days, those presented were at the fourth daily session. Sucrose solution and water drunk were measured over a 4-h test session. NPY-induced sucrose drinking was measured 2 h after NPY injected ICV (control saline animals drunk 3.9± 0.7 g, n = 15). Ethanol and water drunk were estimated after 6 h during 4 days, those presented were at the fourth daily session

SR 141716 mg/kg	Sucrose intake (rats)					Ethanol consumption (mice)	
	Sucrose pellets	Control regular chow	Sucrose solution	Control water intake	NPY-induced sucrose drinking	Ethanol solution	Control water intake
0	3.1 ± 0.4 (12)	2.9 ± 0.2 (12)	28.3 ± 5.0 (8)	8.6 ± 1.9 (8)	8.1 ± 1.7 (15)	1.9 ± 0.1 (20)	1.1 ± 0.1 (20)
0.1					4.1 ± 0.9† (10)	1.4 ± 0.2 (10)	1.1 ± 0.3 (10)
0.3	2.1 ± 0.2 (12)	3.3 ± 0.2 (12)	16.1 ± 3.9 (6)	6.9 ± 2.0 (7)	3.6 ± 1.2† (10)	1.3 ± 0.2 (10)	1.1 ± 0.3 (10)
1	1.6 ± 0.2** (12)	2.9 ± 0.3 (12)	7.4 ± 2.8** (6)	6.5 ± 1.6 (7)	1.2 ± 0.4†† (10)	1.1 ± 0.2** (10)	1.3 ± 0.1 (10)
3	1.0 ± 0.4** (12)	2.9 ± 0.3 (12)	8.6 ± 2.9** (6)	8.4 ± 0.6 (7)		1.0 ± 0.2** (10)	1.6 ± 0.3 (10)
ANOVA	$F(3,44) = 7.93$ $P < 0.0002$	$F(3,44) = 0.56$ NS	F(3,22) = 6.17 $P < 0.001$ (1)	$F(3,25) = 0.75$ NS	$F(4,55) = 4.54$[a] $P < 0.01$	$F(4,55) = 5.53$ $P < 0.001$	$F(4,55) = 0.41$ NS

(n) number of animals; * $P < 0.05$; ** $P < 0.01$ vs vehicle or $^{\dagger}P < 0.05$; $^{\dagger\dagger}P < 0.01$ vs NPY (Dunnett's t-test after ANOVA)
[a] Control saline rats (n = 15) have been included in the analysis

given SR 141716 at 3 mg/kg the corresponding values were 1.8 ± 0.3 ($P < 0.01$); 1.9 ± 0.2 ($P < 0.01$); 3.3 ± 0.3 (NS); 2.9 ± 0.3 (NS)]. In food non-restricted rats, the intense sucrose drinking that occurs after 1 week of habituation was markedly and dose-dependently reduced by SR 141716 (Table 1); in contrast, in water-deprived rats, water drinking was not affected by SR 141716. Together, these findings show that blockade of CB1 receptors produced an elective and almost complete suppression of spontaneous sucrose intake in both solid and liquid forms and in both Wistar and Sprague-Dawley rat strains. This cannot be accounted for by SR 141716-induced enhanced neophobia, since rats were tested after habituation towards sucrose pellets or sucrose solution. As shown in Table 1, SR 141716 (which is devoid of affinity for NPY1 receptors) antagonized the enhancement of sucrose ingestion induced by NPY, a neuropeptide the excessive function of which has been associated with abnormal feeding behaviour and obesity (Erickson et al. 1996). Although the density of CB1 receptors in hypothalamus is not as high as that found in other brain areas (Herkenham et al. 1990), the results suggest that a reduction in NPY-hypothalamic function could be one of the mechanisms involved in the suppression of sucrose craving associated with the blockade of CB1 receptors. Whatever the mechanisms involved, these observations are consonant with the reported ability of Δ^9-THC and derivatives to enhance the preference for sweet calories (Sofia and Knobloch 1976). This, together with the fact that the doses of SR 141716 active in the present study are in the range known to block the characteristic in vivo effects of cannabinoid receptor agonists (Rinaldi-Carmona et al. 1994 ; Pério et al. 1996), supports the notion that an endogenous cannabinoid tone could operate to intensify the incentive properties of sucrose. A similar hypothesis has been proposed for chocolate craving following the discovery of the presence of anandamide in chocolate (di Tomaso et al. 1996). Remarkably, SR 141716 also selectively and significantly reduced ethanol ingestion in C57BL/6 mice (Table 1), a strain known for its genetic predisposition for ethanol consumption (Ng and George 1994). This antagonism was found in the 0.3–3 mg/kg dose range, was maintained upon repeated administration, and was observed whether mice were naive (not shown) or familiarized towards ethanol. In this strain of mice, water intake was not modified by SR 141716 up to 3 mg/kg (Table 1). These results suggest that endogenous cannabinoid tone also plays a critical role in the control of ethanol intake.

In conclusion, the results obtained on both sucrose intake and ethanol consumption suggest for the first time that the activation of an endogenous cannabinoid system may alter the appetitive value of ingested substances an hypothesis consistent with the evidence in favor of a facilitatory effect of cannabinoid agonists on brain reward circuits (Gardner and Lowinson 1991; Trojniar and Wise 1991). Thus, cannabinoid receptor antagonists may have potential for the treatment of carbohydrate craving and ethanol abuse.

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