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Acute or chronic effects of cannabinoids on spontaneous or pharmacologically induced yawning in rats

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Abstract

Yawning is a reflex or event that is not fully understood. It is controlled by many neurotransmitters and neuropeptides and can be induced pharmacologically by cholinergic or dopaminergic agonists. Amongst their many actions, cannabinoids acting on cannabinoid (CB_1 or CB_2) receptors can alter cholinergic and/or dopaminergic activity. This study examined the effects of Δ^8-tetrahydrocannabinol (Δ^8-THC) administered acutely (2.5 mg/kg intraperitoneally [ip], 15 min before test) or chronically (5 mg/kg for 30 days followed by 24 h or 7 days of discontinuation) on yawning induced by pilocarpine, a cholinergic agonist (0, 1, 2, 4 or 8 mg/kg ip), or apomorphine, a dopaminergic agonist (0, 20, 40 or 80 μg/kg subcutaneously [sc]). Acute effects of different doses of Δ^9-tetrahydrocannabinol (Δ^9-THC: 0, 0.5, 1.25 or 2.5 mg/kg ip) on yawning induced by pilocarpine (2 mg/kg ip) or apomorphine (40 μg/kg sc) were also investigated. Both pilocarpine and apomorphine produced yawning in a dose-related manner. Acute administration of Δ^8-THC and Δ^9-THC significantly reduced yawning induced by both pilocarpine and apomorphine. Chronic administration of Δ^8-THC did not change yawning induced by either agonist 24 h or 7 days after discontinuation of Δ^8-THC. However, a high frequency of spontaneous yawning was observed 7 days after Δ^8-THC discontinuation. These results suggest that cannabinoid agonists inhibited yawning induced by cholinergic or dopaminergic agonists. In addition, the increased frequency of spontaneous yawning following cessation of chronic administration of a cannabinoid agonist may be of importance as a withdrawal sign for these drugs.

Keywords: Cannabinoids; Δ^8-THC; Δ^9-THC; Yawning; Pilocarpine; Apomorphine

1. Introduction

In the past decade, the results of many studies have greatly increased the understanding of the physiology and pharmacology of cannabinoids in the central and peripheral nervous systems (see Nakamura-Palacios et al., 1999, for a review). For example, cannabinoid CB_1 and CB_2 receptors (Matsuda et al., 1990; Munro et al., 1993) and a subtype CB_{1A} (Shire et al., 1995), have been characterized (Devane et al., 1988), cloned (Matsuda et al., 1990; Marx, 1990) and the second messenger systems identified (Childers et al., 1992; Martin et al., 1994; Childers and Deadwyler, 1996). Anandamide, 2-arachidonyl glycerol, homo-γ-linolenylethanolamide, 7,10,13,16-docosatetraenyl-ethanolamide, mead ethanolamide and palmitoylethanolamide have been proposed as endogenous ligands for cannabinoid receptors (Devane et al., 1992; Stella et al., 1997; Pertwee, 1997). The availability of cannabinoid antagonists selective for the CB_1 receptor, SR141716A, and CB_2 receptor, SR144528 (Rinaldi-Carmona et al., 1994; Calignano et al., 1997), has greatly facilitated studies on the physiological functions of cannabinoid systems. Additionally other antagonists such as WIN 56,098, 6-bromopravodoline (WIN 54,461), 6-iodopravadoline (AM630), LY320135 (Hosohata et al., 1997; Pertwee, 1997; Felder et al., 1998), have also been synthesized and characterized.

Cannabinoids affect the actions and release of many neurotransmitters, including acetylcholine (ACh) and dopamine (DA) (Pertwee, 1990). Recent studies have demon-

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strated that cannabinoids act at presynaptic CB_1 receptors to inhibit ACh release in ileal myenteric plexus-longitudinal smooth muscle preparations (López-Redondo et al., 1997; Izzo et al., 1998; Coutts and Pertwee, 1998; see Izzo et al., 2001, for a review), the hippocampus and the medial–prefrontal cortex (Carta et al., 1998; Gifford and Ashby, 1996; Gessa et al., 1998a). SR 141716A antagonizes the inhibition of hippocampal ACh release produced by cannabinoid agonists, suggesting that the effects of cannabinoids on learning and memory depend on a CB_1 receptor-mediated inhibition of ACh release in the hippocampus (Gessa et al., 1997). Also, the combination of SR 141716A or Δ^9-tetrahydrocannabinol (Δ^9-THC) with scopolamine produced larger disruptive effects on a repeated-acquisition procedure in squirrel monkeys than those observed when either Δ^9-THC or scopolamine was administered alone, indicating that either a CB_1-receptor agonist or antagonist can alter the disruptive effects of scopolamine on learning in squirrel monkeys (Nakamura-Palacios et al., 2000).

In contrast to the inhibition of ACh release, the stimulation of CB_1 receptors produces an activation of mesoprefrontal or mesolimbic dopaminergic transmission (French, 1997; Diana et al., 1998; Gessa et al., 1998b; Mascia et al., 1999). Because these dopaminergic circuits are involved in the reinforcing effects of most drugs of abuse, the enhanced dopaminergic activity might underlie the reinforcing and abuse properties of marijuana (Diana et al., 1998; Ameri, 1999). Additionally, the disruptive effects of cannabinoids on cognitive processes might be related to the activation of dopaminergic transmission in the prefrontal cortex (Diana et al., 1998).

Adversely, the synthetic cannabinoid agonist, HU 210, antagonized motor hyperactivity and stereotypical behavior elicited by cocaine and a DA receptor agonist, CQP 201–403 (Ferrari et al., 1999). HU 210 also antagonized penile erection and stretching–yawning elicited by dopaminergic D_2/D_3 agonists, B-HT 920 and 7-OH-DPAT, in a manner similar to that produced by a dopaminergic D_2 antagonist, (−) eticlopride (Ottani et al., 2002). Additionally, an intracerebroventricular administration of an anandamide tranport inhibitor *N*-(4-hydroxyphenyl)-arachidonamide (AM404), which causes anandamide to accumulate in the central nervous system, produced a mild and slow-developing hypokinesia and reduced the stimulation of motor behaviors elicited by the selective D_2 family receptor agonist quinpirole (Beltramo et al., 2000). Therefore, it seems that cannabinoid agonists can both increase and decrease dopaminergic activity.

Yawning is a reflex or stereotyped event exhibited by all mammals and vertebrates (Chouard and Bigot Massoni, 1990; Argiolas and Melis, 1998). It seems to be a brain stem arousal reflex with both peripheral and central loops subserving reversal of brain hypoxia or hypoxemia (Aloe, 1994), probably related to an effort to keep vigilance (Blin et al., 1991). Its mechanisms and functional role are not entirely known (Chouard and Bigot Massoni, 1990; Aloe, 1994; Argiolas and Melis, 1998). It seems to be centrally linked with the dopaminergic system in a D_1–D_2 cooperation (Blin et al., 1991) and the cholinergic system as the effector pathway (Yamada and Furukawa, 1980; Tufik et al., 1987; Blin et al., 1991) for the dopaminergic–cholinergic linked neural mechanism (Ushijima et al., 1988; Kimura et al., 1996).

However, many other neurotransmitters and neuropeptides, such as excitatory amino acids, serotonin, gama-aminobutyric acid, noradrenaline, nitric oxide, adrenocorticotropic hormone related peptides, oxytocin and opioid peptides, are also involved in the central control of yawning (Blin et al., 1991; Aloe, 1994; Argiolas and Melis, 1998).

The studies reviewed above demonstrate that cannabinoids can alter transmission mediated by both dopaminergic and cholinergic pathways, both of which are involved in the yawning response. Because cannabinoids are known to alter responses mediated by these neurotransmitter systems, the current study was carried out to examine the effects of acute and chronic treatments with cannabinoid agonists on the spontaneous yawning response as well as that produced by a cholinergic and dopaminergic agonists in rats.

2. Method

2.1. Subjects

Male Wistar rats (Psychobiology Department, UNIFESP, or Pharmacology Department, UFSC) 2.5–3 months old weighing between 250 and 300 g were used. The subjects were maintained on a 12-h light–dark cycle (lights on at 7:00 a.m. to 7:00 p.m.) in a temperature-controlled animal room. They were housed in wire cages in groups of three subjects. The care and use of all subjects in this study followed the guidelines for experimental animal utilization that are in conformity with international principles for research involving animals.

2.2. Drugs

Δ^8-THC and Δ^9-THC were provided by the National Institute on Drug Abuse (DHHS, NIDA, NC) and the National Institute of Mental Health (NIMH, MD) and were kept refrigerated in darkness until just prior to use. Suspensions for injection were prepared by the method of Carlini and Kramer (1965). The alcohol in which the drug was dissolved was evaporated and the residue resuspended in 0.9% NaCl solution containing one drop of Tween-80 per 10 ml. The fine colloidal suspension that resulted had 2.5 mg of the Δ^8-THC residue/ml or 0.5, 1.25 or 2.5 mg of Δ^9-THC residue/ml for acute administrations and 5 mg/ml of Δ^8-THC for chronic administration. Pilocarpine HCl and apomorphine HCl (Sigma Chemical) were dissolved in distilled water to give concentrations of 1, 2, 4 and 8 mg/ml for pilocarpine HCl and 20, 40 and 80 μg/ml for apomorphine

HCl (Sigma Chemical). All drugs were administered in a volume of 0.1 ml/100 g body weight. Δ^8-THC, Δ^9-THC, saline (controls) and pilocarpine were administered by the intraperitoneal (ip) route and apomorphine subcutaneously (sc).

2.3. Procedure

On the day of the experiment the animals were brought to the laboratory and placed in individual wire cages. During the 30-min period immediately following the administration of pilocarpine, apomorphine or saline, the number of yawns was counted as the total number of deep inspirations made through a wide-open mouth associated with and without stretching. All measurements were taken in the afternoon, beginning at 1:30 p.m.

For acute treatments, Δ^8-THC (2.5 mg/kg) or saline was administered 15 min before saline or pilocarpine (0, 1, 2, 4 or 8 mg/kg) or apomorphine (0, 20, 40 or 80 μg/kg). Δ^9-THC (0.5, 1.25 or 2.5 mg/kg) or saline was administered 15 min before saline or pilocarpine (2 mg/kg) or apomorphine (40 μg/kg).

For chronic treatment, Δ^8-THC (5 mg/kg) or saline was administered once a day in the afternoon for 30 days. Twenty-four hours and 7 days later, the effects of saline, pilocarpine (1 or 2 mg/kg) or apomorphine (20 or 40 μg/kg) were determined. This procedure was conducted to determine if the chronic treatment with a cannabinoid agonist resulted in changes in the sensitivity to either pilocarpine or apomorphine in a manner similar to that produced by a chronic treatment with a DA antagonist seen at 7 days after cessation of treatment (Gianutsos et al., 1974; Gianutsos and Lal, 1976).

2.4. Data analyses

Data are presented as mean ± S.E.M. number of yawns recorded during 30 min after drug or saline administration. Two-way analysis of variance for independent measures followed by Tukey's test were used in the comparison of data obtained among different doses of pilocarpine or apomorphine following saline or Δ^8-THC, and also for data obtained after chronic treatment with Δ^8-THC or saline. One-way analyses of variance for independent measures followed by Tukey's test were used to compare the effects of different doses of Δ^9-THC combined with pilocarpine or apomorphine. In all analyses, an alpha level of two-tailed $P<.05$ was employed.

3. Results

Effects of pilocarpine combined with saline or Δ^8-THC are shown in the upper panel of the Fig. 1. Two-way analysis of variance showed statistically significant differences between treatments with saline or Δ^8-THC

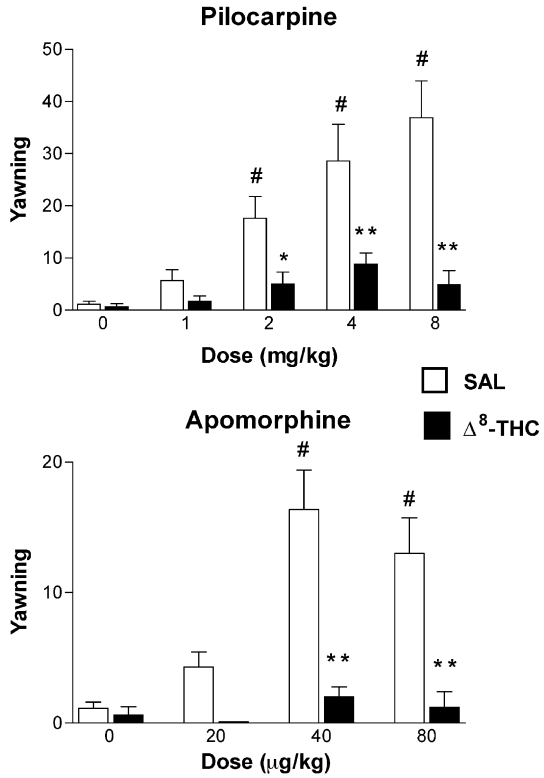

Fig. 1. Effects of acute administration of Δ^8-THC (2.5 mg/kg ip) on yawning induced by different doses of pilocarpine (upper panel) or apomorphine (lower panel). The bars and error symbols represent the mean (± S.E.M.) number of yawns seen in the 30-min observation period. * $P<.05$ compared to the same dose of pilocarpine preceded by saline; ** $P<.01$ compared to the same dose of pilocarpine or apomorphine preceded by saline; # $P<.01$ compared to the control saline–saline (0).

[$F(1,86)=30.98$, $P<.0001$] and among different doses in each treatment [$F(4,86)=10.22$, $P<.0001$]. There was also a significant interaction between treatment and doses [$F(4,86)=5.23$, $P=.0008$]. Pilocarpine produced yawning in a dose-related manner. Subjects treated with doses of 2, 4 or 8 mg/kg of pilocarpine preceded by saline showed a higher mean number of yawns ($P<.01$) as compared to the control (saline–saline). Subjects receiving Δ^8-THC (2.5 mg/kg) prior to pilocarpine showed a significant lower mean number of yawns ($P<.05$ for dose of 2 mg/kg and $P<.01$ for doses of 4 or 8 mg/kg) induced by pilocarpine as compared to animals treated with pilocarpine preceded by saline. It is of interest to note that increasing doses of pilocarpine were not able to surmount the antagonism produced by Δ^8-THC.

Effects of apomorphine combined with saline or Δ^8-THC are shown in the lower panel of Fig. 1. The comparison between treatments [$F(1,64)=45.29$, $P<.0001$] and among doses [$F(3,64)=11.82$, $P<.0001$] was statistically significant. There was also a significant interaction between treatment and doses [$F(3,64)=7.85$, $P=.0002$]. Apomorphine also produced yawning in a dose-related manner. Subjects treated with 40 or 80 μg/kg of apomorphine showed a significant higher mean number of yawns ($P<.01$) com-

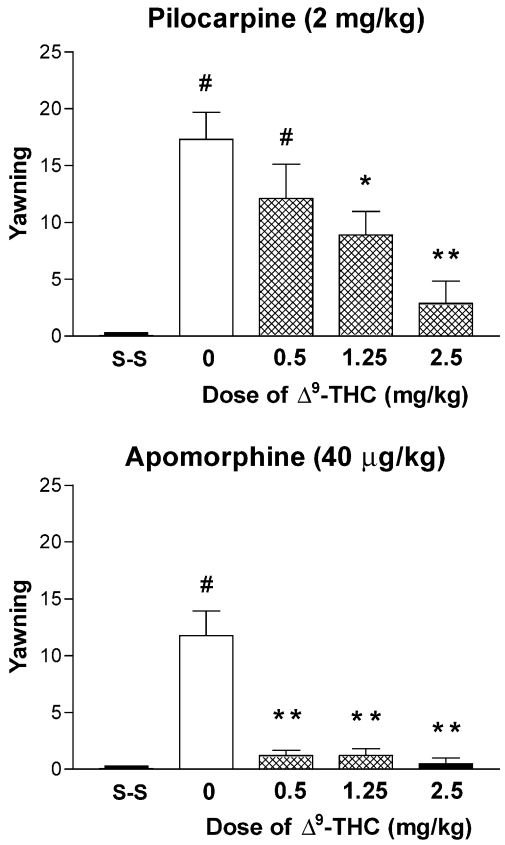

Fig. 2. Effects of acute administration of different doses of Δ^9-THC on yawning induced by pilocarpine (2 mg/kg ip) (left panel) or apomorphine (40 μg/kg sc) (right panel). The bars and error symbols represent the mean (±S.E.M.) number of yawns seen in the 30-min observation period. * $P<.05$ compared to pilocarpine preceded by saline (0); ** $P<.01$ compared to pilocarpine or apomorphine preceded by saline (0); # $P<.01$ compared to the control saline–saline (S-S).

pared to the control (saline–saline). Pretreatment with of Δ^8-THC (2.5 mg/kg) completely abolished yawning induced by all doses of apomorphine.

Fig. 2 shows that Δ^9-THC dose-dependently reduced yawning induced by pilocarpine (2 mg/kg). In contrast, the yawning induced by apomorphine (40 μg/kg) was completely abolished by all doses of Δ^9-THC. As expected, pilocarpine 2 mg/kg and apomorphine 40 μg/kg preceded by saline significantly induced a higher mean number of yawns ($P<.01$) compared to the control (saline–saline) to a very similar extent. There were statistically significant differences among the effects of different doses of Δ^9-THC preceding pilocarpine [$F(43,4)=9.32$, $P<.0001$] or apomorphine [$F(43,4)=16.06$, $P<.0001$]. Doses of 1.25 or 2.5 mg/kg of Δ^9-THC combined with pilocarpine produced significantly less yawning ($P<.05$ and 0.01, respectively) compared to the effect of pilocarpine preceded by saline. All doses of Δ^9-THC (0.5, 1.25 and 2.5 mg/kg) equally diminished ($P<.01$) yawning induced by apomorphine. There were no statistically differences in the comparison among different doses of Δ^9-THC followed by saline compared to control (saline–saline) (Table 1).

Table 1
Effects of acute administration of Δ^9-THC combined with saline on yawning

Drug combination	Yawning
Saline–saline	0±0 (8)
Δ^9-THC	
0.5 mg/kg–saline	0.5±0.34 (6)
1.25 mg/kg–saline	0±0 (8)
2.5 mg/kg–saline	0±0 (4)

Data are presented as mean±S.E.M. (number of subjects).

Table 2
Effects of chronic administration of Δ^8-THC (5 mg/kg) on yawning induced by pilocarpine or apomorphine, 24 h or 7 days after the last drug administration

Acute treatment	Period after chronic treatment	Yawning chronic treatment (30 days)	
		Saline	Δ^8-THC
Saline	24 h	2.13±1.07 (8)	0.88±0.52 (8)
	7 days	1.53±0.44 (17)	5.53±1.31 (17)**
Pilocarpine			
1 mg/kg	7 days	10.00±2.95 (8)[a]	7.63±2.89 (8)
2 mg/kg	24 h	13.13±3.17 (8)[b]	11.38±4.43 (8)[b]
	7 days	15.38±2.57 (8)[a]	17.88±3.70 (8)[a]
Apomorphine			
20 μg/kg	7 days	10.13±2.44 (8)[a]	9.13±1.44 (8)
40 μg/kg	24 h	19.43±6.23 (7)[b]	18.63±5.04 (8)[b]
	7 days	23.13±4.8 (8)[a]	19.88±4.63 (8)[a]

Note the high expression of spontaneous (i.e., nonpharmacologically induced) yawning in animals treated chronically with Δ^8-THC compared to those treated with saline. Data are presented as mean±S.E.M. number of yawns (number of subjects).

[a] $P<.05$ compared to their respective control (saline–saline or Δ^8-THC–saline) 7 days after chronic treatment).

[b] $P<.05$ compared to their respective control (saline–saline or Δ^8-THC–saline) 24 h after chronic treatment).

** $P<.01$ compared to saline+saline 7 days after chronic treatment.

Chronic administration of Δ^8-THC for 30 days did not change yawning induced by either of the doses of pilocarpine (1 or 2 mg/kg) or apomorphine (20 or 40 μg/kg), 24 h or 7 days after the last drug administration (Table 2). However, it is of interest to note that at 7 days after cessation of Δ^8-THC treatment, the rate of spontaneous yawning (yawning observed after saline administration) was significantly greater ($P<.01$, Tukey's test) than that seen in subjects treated chronically with saline.

4. Discussion

The dose-dependent yawning induced by cholinergic and dopaminergic agonists was consistent with several studies (Yamada and Furukawa, 1980; Ushijima et al., 1984a; Tufik et al., 1987).

One could argue that what we are calling as yawning might actually be what is described as conditional gaping in rats as a manifestation of the vomiting response, relating our results to an antiemetic effect of cannabinoids. However,

gaping behavior is characterized by a rapid opening and closing of the mouth usually accompanied by chin rubbing, reflecting an aversive response (Sederholm and Södersten, 2002) or a rejection taste reactivity response (Parker and Brosseau, 1990), in contrast to yawning, a slower and wide opening of the mouth, sometimes accompanied by stretching behavior, a pattern of response observed in our study. Additionally, the experimental observation of a conditional rejection reactions in rats usually needs a flavor, which will induce this response through oral infusion, to be previously paired to an emetic drug such as lithium chloride (Pelchat et al., 1982; Parker et al., 2002), a procedure that was not used in our study. Finally, there are very few reports in the literature referring to cholinergic agonists inducing gaping (Rupniak et al., 1983; Salamone et al., 1986; Collins et al., 1993), or apomorphine inducing gaping and this one in large doses, far beyond the dose range employed in our study (Parker and Brosseau, 1990).

According to Yamada and Furukawa (1980) and Ushijima et al. (1984a), yawning induced by pilocarpine is produced centrally, as shown by the observation that yawning induced directly by a cholinergic agonist such as pilocarpine or indirectly by a cholinesterase inhibitor such as physostigmine can be blocked by muscarinic antagonists that penetrate the central nervous system (e.g., scopolamine) but not by those acting only peripherally (e.g., methylscopolamine).

Low doses of apomorphine act preferentially at D_2 presynaptic receptors to cause a reduction in DA release. Considering the inhibitory modulation of DA on ACh release, the reduced release of DA could result in a greater release of ACh, and thereby increase yawning (Yamada and Furukawa, 1980; Ushijima et al., 1984a; Tufik et al., 1987). Centrally acting muscarinic antagonists abolish yawning induced by dopaminergic and cholinergic agonists, whereas dopaminergic antagonists only abolish apomorphine-induced yawning (Yamada and Furukawa, 1980; Ushijima et al., 1984a; Tufik et al., 1987). These observations strongly suggest that the yawning induced by low doses of a dopaminergic agonist is due to an increase in central cholinergic transmission.

In the present study, acutely administered Δ^8-THC or Δ^9-THC decreased yawning induced by pilocarpine in a dose-dependent manner and completely blocked yawning induced by apomorphine. It is likely that these actions involve a cannabinoid modulation of central dopaminergic and/or cholinergic systems, a dopaminergic–cholinergic linked neural mechanism or actions of the cannabinoids at another site distal to that of pilocarpine.

The inhibitory effects of these cannabinoids on pilocarpine-induced yawning cannot be readily explained by the known ability of cannabinoid agonists to inhibit ACh release (López-Redondo et al., 1997; Coutts and Pertwee, 1997; Gifford et al., 1997) or a competitive interaction between the cannabinoid and pilocarpine for occupancy of muscarinic receptors. The cannabinoid agonists produced a dose-dependent inhibition of the actions of pilocarpine that could not be overcome by increasing doses of pilocarpine. This form of antagonism is analogous to that seen with the inhibition of indirectly acting agonists (Black et al., 1980; Barker and Ebersole, 1982) and suggests that the cannabinoids are acting at a site distal to the actions of pilocarpine.

Yawning induced by low doses of apomorphine was much more sensitive to antagonism by both Δ^8-THC and Δ^9-THC than that produced by pilocarpine. Similar to what was observed for pilocarpine, increasing doses of apomorphine did not overcome the inhibition produced by Δ^8-THC. Apomorphine at 40 μg/kg and pilocarpine at 2 mg/kg produced similar increases in yawning. The lowest dose of Δ^9-THC, 0.5 mg/kg, completely abolished the response to apomorphine. In contrast, this dose only produced about a 30% decrease in the yawning produced by pilocarpine. The inhibitory effects of the cannabinoid agonists on the actions of apomorphine appear to be due to an antagonism rather than an enhancement of dopaminergic transmission.

Behavioral effects of apomorphine are biphasic; low doses induce yawning and sedation, probably by presynaptic D_2 autoreceptors activation, whereas higher doses induce stereotypy and hyperactivity, probably by postsynaptic D_1 activation (Yamada and Furukawa, 1980). These behaviors are mutually exclusive (Tufik et al., 1987) in that yawning decreases with increasing doses of apomorphine.

Several studies have shown that cannabinoid agonists can either increase dopaminergic activity or produce a dopaminergic antagonistic-like effect. The antagonistic-like actions of cannabinoid agonists might best explain our results since the apomorphine-induced yawning was the most affected by cannabinoid agonists and no sign of stereotypy or hyperactivity was observed in animals treated with the combination of cannabinoids with apomorphine.

This antagonistic-like action of cannabinoid agonists may involve D_2 dopamine receptor mediation. Beltramo et al. (2000) recently showed that both, anandamide transport inhibitor AM404 and anandamide by itself counteract two characteristic responses mediated by activation of D_2 family receptors, that is, yawning induced by apomorphine in a dose equivalent to the highest one employed in our study (80 μg/kg sc) and quinpirole-induced stimulation of motor behaviors. Because the stimulation of motor behavior elicited by systemic administration of the D_2-like agonist quinpirole was increased by SR 141716A, Giuffrida et al. (1999) suggested that the endocannabinoid system may modulate D_2 dopamine-induced activation of psychomotor activity acting as an inhibitory feedback mechanism to this behavior.

Neurotransmitter or neuromodulator systems other than cholinergic or dopaminergic also have to be considered. According to Argiolas and Melis (1998), activation of the paraventricular nucleus of the hypothalamus by DA, excitatory aminoacids and oxytocin facilitates yawning by releasing oxytocin at extrahypothalamic areas such as the hippocampus, the pons and/or the medulla oblongata that

play a key role in the expression of this behavioral event. The yawning induced by these neurotransmitters or neuropeptides was only antagonized by opioid peptides (Argiolas and Melis, 1998).

There were no changes in the ability of pilocarpine or apomorphine to induce yawning at 24 h or 7 days after cessation of the chronic administration of Δ^8-THC. Previous studies on yawning have shown that rodents treated chronically with haloperidol, a dopaminergic antagonist, exhibited a central hyposensitivity (Dustan and Jackson, 1977) to apomorphine and physostigmine, both of which act via the release of ACh, but not with pilocarpine, a directly acting agonist (Ushijima et al., 1984b). On the other hand, after chronic treatment with muscarinic antagonists such as atropine or scopolamine there was a supersensitivity to physostigmine and pilocarpine (Takeyasu et al., 1979; Ushijima et al., 1984b), but not to apomorphine (Ushijima et al., 1984b). Our results suggest that, unlike their effects on other behaviors, chronic treatment with a cannabinoid agonist does not alter the sensitivity of systems modulating yawning.

Recent studies have shown that the repeated administration of cannabinoid agonists might or might not change the acute effects of some drugs. For example, Ferrari et al. (1999) found that a short-term treatment (7 days) with a cannabinoid agonist, HU 210, did not modify cocaine-induced effects, although it increased locomotor activity and stimulated escape attempts produced by a D_1/D_2 agonist, CQP 201–403. A chronic administration of Δ^9-THC (3 weeks) did not change the effects of amphetamine or heroin in low-responder rats, but it significantly increased the locomotor effects of these drugs in high-responder rats (Lamarque et al., 2001).

Nevertheless, a salient finding of the present study was that animals treated with Δ^8-THC for 30 days showed higher spontaneous yawning 7 days after drug discontinuation compared to animals treated with saline. The latency for this effect of chronic cannabinoid treatment is much less than that observed by Lamarque et al. (2001). In their study, the increased locomotor responses to heroin only occurred in high-responder rats with a latency of 41 days after cessation of treatment for heroin-treated rats. The basis for this difference in latency of presumed withdrawal signs is not known.

The lack of any changes in the sensitivity to pilocarpine or apomorphine after cessation of treatment suggests that the increased spontaneous yawning observed in our study could be due to changes in noncholinergic, nondopaminergic neurotransmitter or neuromodulator systems involved in yawning, such as opioid peptides (Argiolas and Melis, 1998). Endogenous opioid peptides seem to exert an inhibitory control on the yawning response at the paraventricular level (Argiolas and Melis, 1998). The repeated administration of cannabinoid agonists produced a time-related increase in proenkephalin gene expression and mu-opioid receptor activation of G-proteins in the paraventricular nucleus, as well as in other structures such as spinal cord, caudate-putamen, nucleus accumbens, ventromedial nucleus of hypothalamus, and pituitary (Corchero et al., 1997, 1999a,b; Manzanares et al., 1998). It is proposed that the increase in spontaneous yawning observed after cessation of cannabinoid treatment might be related to the loss of an increased tone at mu-opioid receptors. Yawning is one of the nine signs in the diagnostic criteria for opioid withdrawal (DSM-IV-TR, 1994). The possible involvement of an opioid system in spontaneous yawning following cannabinoid withdrawal merits further investigation.

In summary, acute administration of Δ^8-THC or Δ^9-THC significantly reduced yawning induced by cholinergic or dopaminergic agonists. Chronic exposure to the cannabinoid agonist did not change yawning induced by cholinergic or dopaminergic agonists 24 h or 7 days after drug discontinuation. However, an increased spontaneous yawning was observed 7 days after cannabinoid withdrawal. This sign might provide a good behavioral instrument for carrying out studies on cannabinoid withdrawal and/or dependence.

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