Role of the cannabinoid system in the effects induced by nicotine on anxiety-like behaviour in mice

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Abstract

Rationale: Acute behavioural effects and motivational responses induced by nicotine can be modulated by the endocannabinoid system supporting the existence of a physiological interaction between these two systems. *Objectives*: The present study was designed to examine the possible involvement of the cannabinoid system in the anxiolytic- and anxiogenic-like responses induced by nicotine in mice. *Methods*: Animals were only exposed once to nicotine. The acute administration of low (0.05, sc) or high (0.8 mg/kg, sc) doses of nicotine produced opposite effects in the elevated plus-maze, i.e., anxiolytic- and anxiogenic-like responses, respectively. The effects of the pretreatment with the CB1 cannabinoid receptor antagonist, rimonabant (0.25, 0.5 and 1 mg/kg, ip), and the cannabinoid agonist, Δ9-tetrahydrocannabinol (0.1 mg/kg, ip), were evaluated on the anxiolytic- and anxiogenic-like responses induced by nicotine. *Results*: Rimonabant completely abolished nicotine-induced anxiolytic-like effects and increased the anxiogenic-like responses of nicotine, suggesting an involvement of CB1 receptors in these behavioural responses. On the other hand, Δ9-tetrahydrocannabinol failed to modify nicotine anxiolytic-like responses, but attenuated its anxiogenic-like effects. In addition the association of non-effective doses of Δ9-tetrahydrocannabinol and nicotine produced clear anxiolytic-like responses. *Conclusions*: These results demonstrate that the endogenous cannabinoid system is involved in the regulation of nicotine anxiety-like behaviour in mice, and provide new findings to support the use of cannabinoid antagonists in the treatment of tobacco addiction.

Introduction

The pharmacological effects of nicotine are mediated by the activation of nicotinic acetylcholine receptors (nAChRs). These receptors are members of the superfamily of ligand-gated ion channels, and are composed of five membrane subunits that combine to form a functional receptor (Dani, 2001). Human and animal studies have revealed that nicotine modifies anxiety-like behaviour. Thus, smokers report that cigarette consumption reduces anxiety and relieves stress (Pomerleau 1986; Gilbert et al. 1989), which could play an important role in the maintenance of smoking. Both anxiolytic- (Brioni et al. 1994) and anxiogenic-like effects (Cheeta et al. 2001; Ouagazzal et al. 1999; Olausson et al. 2001) of nicotine can be revealed in animal behavioural paradigms, such as the elevated plus maze. Central nAChRs (Brioni et al., 1993) and other heterologous receptors, such as 5-HT_{1A} receptors (Seth et al., 2002) have been reported to be involved in the anxiolytic-like effects induced by nicotine. In addition, we have recently reported the participation of the opioid system in the effects induced by nicotine on anxiety-like behaviour (Balerio et al., 2005). However, the mechanisms by which nicotine modifies anxiety-related behaviour have not been yet completely elucidated.

The endocannabinoid system has been reported to be involved in the modulation of multiple functions within the central nervous system, including locomotion, anxiety, memory, nociception and reward (Dewey, 1986; Hernandez-Tristan et al., 2000; Ledent et al., 1999; Zimmer et al., 1999). Similarly, nicotine administration also modifies locomotion, anxiety, memory, nociception, and produces rewarding effects in several animal models (Clarke and Kumar, 1983; Hildebrand et al., 1999; Marubio et al., 1999; Picciotto et al., 1995). Neuroanatomical studies have shown a high density of CB1 receptors in neurons of the cerebellum, basal ganglia, limbic cortices, hippocampus,

hypothalamus and different nuclei of the extended amygdala (Tsou et al., 1998). Interestingly, an overlapping distribution of CB1 cannabinoid receptors and nAChRs has been reported in several brain structures, such as the hippocampus and the amygdala (Picciotto et al., 2000), which supports the possibility of functional interactions between these two systems. Cannabinoid receptor activation has been reported to modulate the release and turnover of ACh in various brain areas. Thus, cannabinoid agonists enhanced ACh release in the hippocampus, cortex and striatum (Tripathi et al., 1987; Acquas et al., 2000), and decreased ACh turnover in these structures (Revuelta et al., 1978; Tripathi et al., 1987). However, a decrease of ACh release has been also reported in the medial-prefrontal cortex and hippocampus after cannabinoid administration (Gessa et al., 1998). The behavioural and biochemical consequences of the interaction between the cannabinoid agonist Δ9-tetrahydrocannabinol (Δ9-THC) and nicotine are poorly documented in animal models in spite of the current association of these two substances in humans. In mice, nicotine facilitated hypothermia, antinociception, hypolocomotion and anxiolytic-like responses induced by Δ9-THC (Valjent et al., 2002), whereas Δ9-THC decreased somatic and motivational manifestations of nicotine withdrawal (Balerio et al., 2004).

Cannabinoid receptors located in the limbic system participate in regulating a variety of emotional responses (Rodríguez de Fonseca et al. 1996; Valjent et al. 2002) and an endogenous cannabinoid tone seems to be relevant for the physiological control of the emotional behaviour, as revealed by (i) the anxiogenic-like response induced in rats by the CB1 cannabinoid receptor antagonist, rimonabant (Navarro et al. 1997) and (ii) the increase in the basal level of anxiety observed in CB1 knockout mice (Martín et al. 2002).

The aim of the present study was to investigate the possible involvement of the endocannabinoid system in the anxiolytic- and anxiogenic-like responses induced by nicotine. For this purpose, the effects of the pretreatment with the selective CB1 cannabinoid receptor antagonist rimonabant and the cannabinoid agonist Δ9-THC were evaluated on the anxiolytic- and anxiogenic-like responses induced by nicotine in the elevated plus-maze in mice.

Materials and methods

Animals

Male CD1 mice (Charles River, France) weighing 22-24 g at the beginning of the study were housed five per cage in a temperature (21 $\pm$ 1 ºC) and humidity (55 $\pm$ 10%) controlled-room with a 12-h/12-h light-dark cycle (light between 08:00 and 20:00 hours). Food and water were available *ad libitum*. Mice were habituated to their new environment for one week after arrival before starting the experimental procedure. Animal procedures were conducted in accordance with the guidelines of the European Communities Directive 86/609/EEC regulating animal research and approved by the local ethical committee. All experiments were performed with the investigators being blind to the treatment conditions.

Drugs

(-)-Nicotine hydrogen tartrate salt ([-]-1-methyl-2-[3-pyridyl]pyrrolidine) (Sigma Chemical Co., Madrid Spain) was dissolved in physiological saline (0.9% NaCl) and only administered once by subcutaneous route. The selective CB1 receptor antagonist rimonabant (SR141716A) [(N-piperidin-1-yl)-5-(4-chlorophenyl)-1(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxyamide] (Sanofi Recherche, Montpellier, France) was dissolved in a solution of 10% ethanol, 10% cremophor EL and 80% distilled water and administered by intraperitoneal route. Δ9-THC (THC Pharm, Franckfurt, Germany) was dissolved in a solution of 5% ethanol, 5% cremophor EL (Sigma Chemical Co., Madrid Spain) and 90% distilled water and was administered by intraperitoneal route.

Rimonabant (0.25, 0.5 and 1 mg/kg) and Δ9-THC (0.1 mg/kg) were administered, 25 min before nicotine (0.05 and 0.8 mg/kg), respectively, by intraperitoneal route. The doses of nicotine refer to the salt form. All drugs were injected in a volume of 10 ml/kg.

Elevated plus-maze

The elevated plus-maze (Pellow et al., 1985; File 1992) consisted of a black plastic apparatus with four arms (16 X 5 cm) set in a cross from a neutral central square (5 X 5 cm). Two opposite arms were delimited by vertical walls (closed arms), whereas the two other opposite arms had unprotected edges (open arms). The maze was elevated 30 cm above the ground and illuminated from the top (100 lux). At the beginning of the 5-min observation session, each mouse was placed in the central neutral area, facing one of the open arms. The total number of visits to the closed and open arms, and the cumulative time spent in the open and closed arms were then observed on a monitor through a videocamera system (View Point, Lyon, France). Results are expressed as the percentage of the time and number of entries in the open arms which provide the measures of anxiety, and the number of closed arms entries provides the best measure of locomotor activity in this test. Measurements in the open and closed arms were recorded when the mouse moved two forepaws and head into the arm. All the observation sessions started 5 min after the acute injection of nicotine.

Experimental design

Several doses of nicotine have been previously tested to select those which produce anxiolytic- and anxiogenic-like responses (Balerio et al., 2005). First, the effects of rimonabant (0.25, 0.5 and 1 mg/kg) on nicotine-induced anxiolytic- (0.05) and

anxiogenic-like (0.8 mg/kg) responses were evaluated in different experiments (n = 17-20 per group). In a second set of experiments, the effects of Δ9-THC (0.1 mg/kg) on nicotine-induced anxiolytic- (0.05) and anxiogenic-like (0.8 mg/kg) responses were evaluated (n = 18-19 per group). Finally, the effects of the association of sub-threshold doses of nicotine (0.025 mg/kg) and Δ9-THC (0.1 mg/kg) were evaluated on anxiety-like behaviour (n = 15-16). Different groups of drug-naive animals were used for each experiment.

Statistical analysis

The percentage of the time and number of entries in the open arms and the number of entries in the closed arms were analysed using two-way ANOVA with nicotine (saline or nicotine) and cannabinoid ligand (vehicle or cannabinoid ligand) administration as between-subjects factors. When the number of entries in the closed arms was affected, the percentage of time and number of entries in the open arms were analysed using two-way ANCOVA with nicotine (saline or nicotine) and cannabinoid ligand (vehicle or cannabinoid) administration as between-subjects factors, and the number of entries in closed arms as covariate. One-way ANOVA or one-way ANCOVA were then used when appropriate for further analysis. Differences were considered significant if the probability of error was < 5%.

Results

Effects of rimonabant on the anxiolytic-like responses induced by nicotine

The statistical analysis is reported in Table 1. Nicotine (0.05 mg/kg) alone significantly increased the percentage of time spent in the open arms (Fig. 1A,C,E). This effect was abolished by the two higher doses of rimonabant (Fig. 1C, E), but was not significantly altered by the lowest dose (Fig. 1A). Rimonabant alone was without effect at any dose. The same analysis applied to the percentage of open-arms entries, revealed a similar pattern of results (Table 1 and Fig. 1B, D, F). The number of entries in the closed arms was only significantly modified when comparing the two groups of mice treated with the lowest dose of rimonabant (Table 3 and 4). ANCOVA revealed that the effects shown on the percentage of time and entries in the open arms were independent on this effect in the closed arms entries.

Effects of rimonabant on the anxiogenic-like responses induced by nicotine

The statistical analysis is reported in Table 1. Nicotine (0.8 mg/kg) alone significantly decreased the percentage of time spent in the open arms (Fig. 2A, C, E). This effect was increased by the highest dose of rimonabant (Fig. 2E), but was not significantly altered by the two lower doses (Fig. 2A, C). Rimonabant alone was without effect at any dose. The same analysis applied to the percentage of open-arms entries, revealed a similar pattern of results (Fig. 2B, D, F). The number of entries in the closed arms was significantly modified when comparing the two groups of mice treated with rimonabant at the doses of 0.5 and 1 mg/kg (Table 3 and 4). ANCOVA revealed that the effects shown on the percentage of time and entries in the open arms were independent on this effect in the closed arms entries.

Effects of Δ9-tetrahydrocannabinol on the anxiolytic-like responses induced by nicotine

The statistical analysis is reported in Table 2. Nicotine (0.05 mg/kg) alone significantly increased the percentage of time spent in the open arms. This effect was not significantly modified by Δ9-THC (0.1 mg/kg), which did not induce any response when given alone (Fig. 3A). The same analysis applied to the percentage of open-arms entries revealed a similar pattern of results (Fig. 3B). The number of entries in the closed arms was not modified in any experimental group (Tables 3 and 4).

Effects of Δ9-tetrahydrocannabinol on the anxiogenic-like responses induced by nicotine

The statistical analysis is reported in Table 2. Nicotine (0.8 mg/kg) alone significantly decreased the percentage of time spent in the open arms. This effect was significantly reduced by Δ9-THC (0.1 mg/kg), which did not induce any response when given alone (Fig. 4A). Nicotine (0.8 mg/kg) alone significantly decreased the percentage of open-arms entries. However this anxiogenic-like response induced by nicotine was abolished by Δ9-THC (0.1 mg/kg), which did not induce any response when given alone (Fig. 4B). The number of entries in the closed arms was not modified in any experimental group (Tables 3 and 4).

Effects of the association of sub-threshold doses of nicotine and Δ9-tetrahydrocannabinol on anxiety-like behaviour

The statistical analysis is reported in Table 2. Nicotine (0.025 mg/kg) or Δ9-THC (0.1 mg/kg) alone did not modify the percentage of time spent in the open arms. A significant increase of the percentage of time spent in the open arms was observed when nicotine and Δ9-THC were co-administered (Fig. 5A). The same analysis applied to the

percentage of open-arms entries, revealed a similar pattern of results (Fig. 5B). The number of entries in the closed arms was not modified in any experimental group (Tables 3 and 4).

Discussion

The results of this study provide clear evidence that the endogenous cannabinoid system participates in the responses induced by nicotine on anxiety-like behaviour. Thus, the anxiolytic-like effects induced by nicotine were decreased in a dose dependent manner by the CB1 cannabinoid receptor antagonist rimonabant. This antagonist also increased in a dose dependent manner nicotine anxiogenic-like responses. In addition, the cannabinoid agonist Δ9-THC did not modify nicotine anxiolytic-like responses, but attenuated its anxiogenic-like effects. The combination of ineffective doses of nicotine and Δ9-THC produced clear anxiolytic-like responses. In a previous study, several doses of nicotine (from 0.05 to 0.8 mg/kg) were tested in the mouse elevated plus maze under similar experimental conditions. An anxiolytic-like response was induced by the dose of 0.05 mg/kg of nicotine, whereas an anxiogenic-like effect was observed after the administration of 0.8 mg/kg of nicotine (Balerio et al., 2005). These behavioural responses of nicotine were reversed by the selective nicotinic antagonist mecamylamine, revealing the direct involvement of nAChRs (Balerio et al., 2005).

Previous studies have reported behavioural interactions between cannabinoids and nicotine. An early study showed that acute depressant effects of Δ9-THC were potentiated by nicotine co-administration in rats (Pryor et al., 1978). Nicotine also facilitated the effects of Δ9-THC on locomotion, body temperature, nociception and reward (Valjent et al., 2002), whereas nicotine rewarding effects were absent in CB1

knockout mice (Castañé et al., 2002). In addition, Δ9-THC decreased somatic and motivational manifestations of nicotine withdrawal in mice (Balerio et al., 2004). These findings support the presence of physiological interactions between cannabinoid and nicotinic systems (Tripathi et al.,1987; Acquas et al., 2000). In the same line, we observed here that the activity of CB1 cannabinoid receptors is required to induce anxiolytic-like effects by nicotine administration since this response was abolished by rimonabant. On the other hand, rimonabant increased the anxiogenic-like effects of nicotine. In agreement, endogenous cannabinoids seem to participate in the physiological control of emotional behaviour since both acute administration of high doses of rimonabant (Navarro et al., 1997) and the genetic disruption of CB1 receptors (Martín et al., 2002) induced anxiogenic-like responses in rodents. The doses of the CB1 antagonist used in this study were selective enough, as reported by previous studies. Rinaldi-Carmona et al. (1994) have shown the high affinity of rimonabant to CB1 receptor (K_i of 2 nM) and the high selectivity for this receptor since this compound has no affinity (IC_{50}>1 μM) for any other type of receptors or channels investigated. *In vivo* studies demonstrated that low doses of rimonabant (from 0.28 to 1.62 mg/kg) were required to block the pharmacological responses (hypolocomotion, catalepsia, hypothermia and antinociception) induced by the CB1 agonist WIN55212-2 (Rinaldi-Carmona et al, 1994). In agreement, the pharmacological responses (hypolocomotion, hypothermia and antinociception) induced by THC and CP55,940 were also selectively antagonized by similar doses of rimonabant (from 0.3 to 3 mg/kg) (Compton et al, 1996; Lichtman and Martin, 1997)

The association of non-effective doses of nicotine and Δ9-THC produced clear anxiolytic-like responses in the elevated plus maze. Besides, the administration of Δ9-THC did not modify nicotine-induced anxiolytic-like responses, but attenuated its

anxiogenic-like effects. In agreement with this result, the anxiolytic-like responses induced by Δ9-THC in the light-dark box and the open-field were not significantly modified by the co-administration of a non-effective dose of nicotine (Valjent et al., 2002). Both nicotine and Δ9-THC induce complex dose/response effects on anxiety-like behaviour. Thus, anxiolytic- and anxiogenic-like responses can be observed after Δ9-THC or nicotine administration depending on the dose (Costall et al., 1989; Risinger and Oakes, 1995).

To our knowledge, no information has been provided about the acute effects of nicotine on brain endocannabinoids. However, the present results suggest that acute nicotine would be able to enhance endocannabinoid content in the brain structures involved in the control of emotional responses. In agreement, chronic nicotine exposure has been reported to enhance endocannabinoid levels in the brainstem limbic forebrain structures (González et al, 2002). Activation of CB1 receptors by these endocannabinoids could participate in the anxiolytic-like responses induced by nicotine. However, the pharmacological activation of cannabinoid receptors by exogenous administration of Δ9-THC did not enhance nicotine anxiolytic-like effects suggesting that this physiological interaction cannot be potentiated by the simultaneous pharmacological activation of both systems.

On the other hand, the administration of high doses of nicotine or Δ9-THC produces anxiogenic-like effects. Nicotine anxiogenic-like responses could be mediated by the release of some neurotransmitters, such as glutamate and noradrenaline (Brazell et al. 1991; Toth et al. 1992; Sharp and Matta 1993; Fu et al. 1998) which are known to facilitate stress/anxiety-related behaviour (Gray et al. 1994; Guimaraes et al. 1991; Jessa et al. 1995; Onaka et al. 1996). The stimulatory effects of nicotine on corticotrophin releasing factor and ACTH release (Okuda et al. 1993; Bremner et al. 1996; Herman

and Cullinan 1997) could also participate in these anxiogenic-like responses. In the present study, the pharmacological activation of cannabinoid receptors by exogenous administration of a low dose of Δ9-THC (0.1 mg/kg) attenuated nicotine anxiogenic-like effects. Activation of CB1 receptors by such a low dose of Δ9-THC would mimic the effects induced by low doses of nicotine on the endocannabinoid system that are required to produce the anxiolytic-like effects, and counteract by this mechanism nicotine anxiogenic-like responses. In agreement, the association of sub-threshold doses of nicotine and Δ9-THC produced clear anxiolytic-like responses. The synergistic effect observed with these non-effective doses could be explained by the simultaneous activation of CB1 cannabinoid receptors by a low dose of Δ9-THC and endocannabinoid release by nicotine administration.

Anxiety and emotional disorders seem to be an important factor for the establishment and maintenance of tobacco addiction (Pomerleau, 1986). Therefore, the anxiolytic effects induced by nicotine could be a key cause of failure in smoking cessation. Interestingly, rimonabant blocked the anxiolytic-like effects induced by nicotine in mice, at least in our experiments of anxiety (elevated plus maze). Previous studies have also reported that rimonabant blocks the reinforcing effects of nicotine (Cohen et al., 2002) and avoids reinstatement of nicotine seeking in rats (Cohen et al., 2005). Furthermore, rewarding effects of nicotine were abolished in knockout mice deficient in CB1 receptors (Castañé et al., 2002). All these animal studies suggest that the blockade of CB1 receptors represents a very promising target to treat tobacco addiction. In agreement, data from the STRATUS-US trial (smoking cessation in smokers motivated to quit) on the effects of rimonabant in human smokers have shown that chronic rimonabant treatment significantly increase the probability of smoking cessation (Anthenelli and Despres, 2004).

In summary, we provide the first pharmacological evidence for the specific involvement of CB1 cannabinoid receptors in mediating the effects induced by nicotine on anxiety-like behaviour. The elucidation of this new interaction between nicotine and the endogenous cannabinoid system provides interesting insights for better understanding the complex behavioural responses of this drug.

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Figure legends

Figure 1 Rimonabant (RIM) blocks the anxiolytic-like responses induced by nicotine (NIC) in the elevated plus maze. **A, C, E :** percentage of time spent in the open arms; **B, D, F:** percentage of entries in the open arms. RIM (0.25, 0.5 and 1 mg/kg) and NIC (0.05 mg/kg) were administered 25 and 5 min before the test, respectively. Data are expressed as mean ± SEM (n = 17-20 mice for each group). ★★★ $P < 0.001$ when comparing with respective vehicle (VEH) group; ☆☆ $P < 0.01$, ☆☆☆ $P < 0.001$ when comparing with NIC + VEH group (one-way ANOVA).

Figure 2 Rimonabant (RIM) increases the anxiogenic-like responses induced by nicotine (NIC) in the elevated plus maze. **A, C, E:** percentage of time spent in the open arms; **B, D, F:** percentage of entries in the open arms. RIM (0.25, 0.5 and 1 mg/kg) and NIC (0.8 mg/kg) were administered 25 and 5 min before the test, respectively. Data are expressed as mean ± SEM (n = 17-20 mice for each group). ★ $P < 0.05$, ★★★ $P < 0.001$ when comparing with respective vehicle (VEH) group; ☆☆☆ $P < 0.001$ when comparing with NIC + VEH group (one-way ANOVA).

Figure 3 Effects of pretreatment with Δ9-tetrahydrocannabinol (THC) on the anxiolytic-like responses induced by nicotine (NIC) in the elevated plus maze. **A:** percentage of time spent in the open arms; **B:** percentage of entries in the open arms. THC (0.1 mg/kg) and NIC (0.05 mg/kg) were administered 25 and 5 min before the test, respectively. Data are expressed as mean ± SEM (n = 18-19 mice for each group). ★ $P < 0.05$, ★★ $P < 0.01$, ★★★ $P < 0.001$ when comparing with respective vehicle (VEH) group (one-way ANOVA).

Figure 4 Effects of pretreatment with Δ9-tetrahydrocannabinol (THC) on the anxiogenic-like responses induced by nicotine (NIC) in the elevated plus maze. **A:** percentage of time spent in the open arms; **B:** percentage of entries in the open arms. THC (0.1 mg/kg) and NIC (0.8 mg/kg) were administered 25 and 5 min before the test, respectively. Data are expressed as mean ± SEM (n = 18-19 mice for each group).

⋆⋆⋆ $P < 0.001$ when comparing with respective vehicle (VEH) group; ⋆⋆ $P < 0.01$ when comparing with NIC + VEH group (one-way ANOVA).

Figure 5 Effects of the association of sub-threshold doses of nicotine (NIC) and Δ9-tetrahydrocannabinol (THC) on anxiety-like behaviour in the elevated plus maze. **A:** percentage of time spent in the open arms; **B:** percentage of entries in the open arms. THC (0.1 mg/kg) and NIC (0.025 mg/kg) were administered 25 and 5 min before the test, respectively. Data are expressed as mean ± SEM (n = 15-16 mice for each group).

⋆⋆⋆ $P < 0.001$ when comparing with respective vehicle (VEH) group;⋆⋆⋆ $P < 0.001$ when comparing with NIC + VEH group (one-way ANOVA).

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