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ORIGINAL INVESTIGATION

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Involvement of the opioid system in the effects induced by nicotine on anxiety-like behaviour in mice

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Abstract *Rationale:* Recent studies have revealed the participation of the endogenous opioid system in several behavioural responses induced by nicotine including antinociception, rewarding properties, and physical drug dependence. *Objectives:* The present study was designed to examine the possible involvement of the various opioid receptors in the anxiolytic- and anxiogenic-like responses induced by nicotine in mice. *Methods:* The acute administration of low (0.05) or high (0.8 mg/kg) doses of nicotine subcutaneously produced opposite effects in the elevated plus maze, i.e. anxiolytic- and anxiogenic-like responses, respectively. Animals were only exposed once to nicotine. The effects of the pretreatment with the μ-opioid receptor antagonist, β-funaltrexamine (5 mg/kg), the δ-opioid antagonist, naltrindole (2.5 mg/kg) and the κ-opioid antagonist, nor-binaltorphimine (2.5 mg/kg) intraperitoneally were evaluated on the anxiolytic- and anxiogenic-like responses induced by nicotine. *Results:* β-funaltrexamine, but not nor-binaltorphimine or naltrindole, abolished nicotine-induced anxiolytic-like effects, suggesting an involvement of μ-opioid receptors in this behavioural response. On the other hand, naltrindole, but not nor-binaltorphimine or β-funaltrexamine, increased the anxiogenic-like responses of nicotine, suggesting an involvement of δ-receptors in this behavioural effect. *Conclusions:* These results demonstrate that the endogenous opioid system is involved in the effects induced by nicotine on anxiety-like behaviour and provide new findings to further clarify the interaction between these two neurochemical systems.

Keywords Nicotine · Anxiety · Opioid system · Elevated plus maze · Mouse · β-funaltrexamine · Naltrindole · Nor-binaltorphimine

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Introduction

Nicotine is one of the active components in tobacco smoke and plays a major role in tobacco addiction (Mathieu-Kia et al. 2002). This compound produces several behavioural responses including changes on locomotion, nociception, anxiety, learning, memory, rewarding effects, and physical dependence (Decker et al. 1995). 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), and this nicotine effect could play an important role in the maintenance of smoking. Animal studies have revealed that nicotine can produce anxiolytic- (Brioni et al. 1994) and anxiogenic-like effects (Cheeta et al. 2001; Ouagazzal et al. 1999a; Olausson et al. 2001) in the elevated plus maze in rodents. These opposite responses to nicotine are dose-dependent, with lower doses producing anxiolytic-like effects and higher doses inducing anxiogenic-like effects, but other factors such as the strain and the baseline anxiety level could also be of relevance. The anxiolytic-like effects induced by nicotine in the elevated plus maze were blocked by mecamylamine, but not by the peripheral nicotinic antagonist hexametonium, suggesting that central nicotinic receptors mediate this response (Brioni et al. 1993). However, the mecamylamine/hexametonium comparison is a poor test of central vs peripheral nAChR involvement, because the in vivo affinity of hexametonium in rodents is not well established. On the other hand, nicotine increases 5-hydroxytryptamine (5-HT) release in the cortex, striatum, hippocampus, dorsal raphe nucleus, hypothalamus, and spinal cord, which involves a stimulation of 5-HT_{1A} receptors in some of these structures. The 5-HT_{1A} receptors in the dorsal raphe nucleus participate in the anxiolytic effects of nicotine, and these receptors in the dorsal hippocampus

and lateral septum are involved in its anxiogenic effects (Seth et al. 2002). However, the mechanisms by which nicotine modifies anxiety-related behaviour have not yet been completely elucidated.

The endogenous opioid system has been suggested to participate in several central effects of nicotine (Balfour 1982). Thus, nicotine induces the release of endogenous opioid peptides in several brain areas in rodents (Pierzchala et al. 1987; Dhatt et al. 1995). In humans, cigarette consumption increases plasma levels of β-endorphin (Pomerleau et al. 1983), and endogenous opioids seem to participate in the reinforcement of smoking since the opioid antagonists naloxone and naltrexone modulate cigarette consumption and subjective pleasure derived from smoking (Karras and Kane 1980; Wewers et al. 1998). Furthermore, several behavioural and physiological effects, including antinociception, rewarding properties, and physical dependence (Decker and Meyer 1999; Hildebrand et al. 1999; Watkins et al. 2000), are shared by nicotine and opioids. Opioid compounds induce their pharmacological effects by activating three different receptors, μ-, δ-, and κ-opioid receptors (Thompson et al. 1993; Mansour et al. 1995). Previous studies using knockout mice have revealed the involvement of μ-opioid receptors (Berrendero et al. 2002) and peptides derived from preproenkephalin (Berrendero et al. 2005) in the antinociceptive effects, rewarding properties and physical dependence induced by nicotine. However, other animal studies using the nonselective opioid receptor antagonist naltrexone have not revealed the link between the opioid system and nicotine in the rewarding effects of this drug (Corrigall and Coen 1991; Mathieu-Kia et al. 2002). Several studies support a role for the opioid receptors in regulating baseline anxiety states and related behaviour. Thus, knockout mice lacking δ-opioid receptor (Filliol et al. 2000) and mice lacking preproenkephalin (König et al. 1996) showed higher anxiety in several behavioural models. These findings support the notion that tonic activation of δ-receptors by endogenous preproenkephalin-derived peptides positively modulates anxiety states. In contrast, mice lacking μ-opioid receptors present a decreased anxiety-like behaviour, suggesting an opposite role of these receptors in the basal control of anxiety (Filliol et al. 2000).

The aim of the present study was to investigate the possible involvement of μ-, δ-, and κ-opioid receptors in the anxiolytic- and anxiogenic-like responses induced by nicotine. For this purpose, the effects of the pretreatment with selective antagonists of these opioid receptors 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-controlled (21±1°C) and humidity-controlled (55±10%) room with a 12-h/12-h light–dark cycle (light between 0800 and 2000 h). Food and water were available ad libitum. Mice were habituated to their new environment for 1 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) and mecamylamine hydrochloride (Sigma Chemical Co., Madrid, Spain) were dissolved in physiological saline (0.9% NaCl) and only administered once by subcutaneous route. The μ-opioid receptor antagonist, β-funaltrexamine, the δ-opioid receptor antagonist, naltrindole, and the κ-opioid receptor antagonist, nor-binaltorphimine (Sigma Chemical Co.), were dissolved in physiological saline (0.9% NaCl). Mecamylamine (1 mg/kg) was administered 25 min before nicotine (0.05 and 0.8 mg/kg); naltrindole (2.5 mg/kg), nor-binaltorphimine (2.5 mg/kg), and β-funaltrexamine (5 mg/kg) were administered, 25 min, 55 min, and 24 h 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. The preinjection times for the three opioid receptor antagonists were selected considering their receptor selectivity and pharmacokinetics properties. Thus, β-funaltrexamine has antinociceptive actions that were of short duration and that appeared to be mediated by κ-receptor interaction. In contrast, the antagonist actions of β-funaltrexamine were of remarkably long duration and were selective toward μ-opioid receptors 24 and 48 h after administration (Tsuda et al. 1996; Ward et al. 1982). On the other hand, naltrindole has been reported to produce a dose-dependent attenuation of swim stress-induced antinociception when administered 30 min before the test (Jackson et al. 1989). With regard to nor-binaltophimine, Endoh et al. (1992) have observed that the κ-antagonistic action gradually increased, reaching a plateau 2 h after administration. At the same time, the μ-antagonistic action of nor-binaltophimine declined and dissapeared 2 h after administration.

Elevated plus maze

The elevated plus maze (Pellow et al. 1985; File 1992) consisted of a black plastic apparatus with four arms (16×5 cm) set in a cross from a neutral central square (5×5 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 lx). 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. 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 plan

In a preliminary study, several doses of nicotine were tested to select those which produce anxiolytic- and anxiogenic-like responses. For this purpose, mice (n=9–10 per group) received saline or nicotine at the doses of 0.05, 0.1, 0.2, 0.4, and 0.8 mg/kg. The effects of mecamylamine (1 mg/kg) (n=17–20 per group), β-funaltrexamine (5 mg/kg), naltrindole (2.5 mg/kg), and nor-binaltorphimine (2.5 mg/kg) on nicotine-induced anxiolytic- (0.05) and anxiogenic-like (0.8 mg/kg) responses were then evaluated in different experiments (n=26–28 per group). A different antagonist and a single nicotine dose was tested in each experiment by using a 2×2 saline-controlled design. Different groups of drug-naive animals were used for each experiment.

Statistical analysis

In the preliminary study, the percentage of the time and number of entries in the open arms were analysed using one-way ANOVA followed by a Dunnett's post hoc test.

In the second set of experiments, the percentage of the time and number of entries in the open arms were analysed using two-way ANOVA with agonist (saline or nicotine) and antagonist (opioid antagonist or saline) administration as between-subjects factors. One-way ANOVA was then used when appropriate for further analysis. Differences were considered significant if the probability of error was <5%. The level of significance was p<0.025 for all cases after applying Bonferroni's correction.

Results

Dose-dependent effects of nicotine in the elevated plus maze

One-way ANOVA revealed a significant effect of nicotine dose on the percentage of time spent [F(5,52)=9.022, p<0.001] and percentage of entries in the open arms [F(5,52)=5.875, p<0.001]. Dunnett's post hoc test revealed that nicotine (0.05 mg/kg) significantly increased the percentage of time spent in the open arms (p<0.05) compared to vehicle indicating an anxiolytic-like effect. On the contrary, nicotine (0.8 mg/kg) induced a significant decrease in the percentage of time spent (p<0.05) and percentage of entries in the open arms (p<0.05) compared to vehicle indicating an anxiogenic-like response (Table 1).

Mecamylamine blocks the anxiolytic-like responses induced by nicotine

Two-way ANOVA calculated for the percentage of time spent in the open arms showed a significant effect of nicotine treatment, antagonist treatment, and a significant interaction between these two factors (Table 2). One-way ANOVA revealed that acute nicotine (0.05 mg/kg) alone increased the percentage of time spent in the open arms. This effect was blocked by mecamylamine, which did not induce any response when given alone (Table 2 and Fig. 1A). The same analysis of the percentage of open-arms entries revealed a similar pattern of results (Table 2 and Fig. 1B).

Mecamylamine blocks the anxiogenic-like responses induced by nicotine

Two-way ANOVA calculated for the percentage of time spent in the open arms showed a significant effect of nicotine treatment, antagonist treatment, and a significant interaction between these two factors (Table 2). One-way ANOVA revealed that acute nicotine (0.8 mg/kg) alone decreased the percentage of time spent in the open arms. This effect was blocked by mecamylamine, which did not produce any response when given alone (Table 2 and Fig. 2A). The same analysis of the percentage of open-arms entries revealed a similar pattern of results (Table 2 and Fig. 2B).

Effects of β-funaltrexamine on the anxiolytic-like responses induced by nicotine

Two-way ANOVA calculated for the percentage of time spent in the open arms showed a significant effect of nicotine treatment, no effect of antagonist treatment, and a significant interaction between these two factors (Table 2). One-way ANOVA revealed that acute nicotine (0.05 mg/kg) alone increased the percentage of time spent in the open

Table 1 Effects of nicotine on anxiety-like behaviour

Nicotine (mg/kg)	Percent time (open arms)	Percent entries (open arms)
0	20.0±2.3	41.0±1.5
0.05	34.0±2.6[a]	47.4±2.8
0.1	31.4±3.3	48.6±2.4
0.2	25.9±2.9	45.3±2.2
0.4	15.9±2.4	40.0±3.1
0.8	8.0±2.4[a]	30.6±3.1[a]

Data are shown as mean±SEM of nine to ten mice per group
[a]p<0.05 compared to vehicle (Dunnett's test)

Table 2 Effects of mecamylamine, β-funaltrexamine, naltrindole, and nor-binaltorphimine on the anxiolytic- and anxiogenic-like responses of nicotine

	Two-way ANOVA						One-way ANOVA					
	ANT treatment		NIC treatment		Interaction		VEH–VEH vs VEH–NIC		ANT–VEH vs ANT–NIC		VEH–NIC vs ANT–NIC	
	F value	*P* value	*F* value	*P* value	*F* value	*P* value	*F* value	*P* value	*F* value	*P* value	*F* value	*P* value
Anxiolytic-like responses												
Mecamylamine												
Percent time	$F(1,72)=16.970$	<0.001	$F(1,72)=16.613$	<0.001	$F(1,72)=44.335$	<0.001	$F(1,36)=63.982$	<0.001	$F(1,36)=3.033$	NS	$F(1,38)=70.355$	<0.001
Percent entries	$F(1,72)=10.506$	<0.001	$F(1,72)=5.651$	<0.025	$F(1,72)=20.319$	<0.001	$F(1,36)=18.282$	<0.001	$F(1,36)=3.225$	NS	$F(1,38)=33.369$	<0.001
β-funaltrexamine												
Percent time	$F(1,107)=4.996$	NS	$F(1,107)=5.202$	<0.025	$F(1,107)=7.456$	<0.01	$F(1,54)=14.453$	<0.001	$F(1,53)=0.089$	NS	$F(1,53)=9.711$	<0.01
Percent entries	$F(1,107)=1.473$	NS	$F(1,107)=4.273$	NS	$F(1,107)=9.411$	<0.01	$F(1,54)=16.065$	<0.001	$F(1,53)=0.422$	NS	$F(1,53)=8.338$	<0.01
Naltrindole												
Percent time	$F(1,107)=1.301$	NS	$F(1,107)=20.564$	<0.001	$F(1,107)=0.264$	NS	$F(1,53)=15.885$	<0.001	$F(1,54)=6.746$	<0.025	$F(1,53)=0.150$	NS
Percent entries	$F(1,107)=0.001$	NS	$F(1,107)=18.321$	<0.001	$F(1,107)=2.742$	NS	$F(1,53)=24.903$	<0.001	$F(1,54)=2.687$	NS	$F(1,53)=1.570$	NS
Nor-binaltorphimine												
Percent time	$F(1,105)=1.135$	NS	$F(1,105)=26.980$	<0.001	$F(1,105)=0.012$	NS	$F(1,53)=15.072$	<0.001	$F(1,52)=12.255$	<0.001	$F(1,54)=0.395$	NS
Percent entries	$F(1,105)=4.771$	NS	$F(1,105)=15.400$	<0.001	$F(1,105)=1.399$	NS	$F(1,53)=17.712$	<0.001	$F(1,52)=2.951$	NS	$F(1,54)=6.584$	<0.025
Anxiogenic-like responses												
Mecamylamine												
Percent time	$F(1,66)=42.608$	<0.001	$F(1,66)=41.531$	<0.001	$F(1,66)=14.833$	<0.001	$F(1,33)=72.128$	<0.001	$F(1,33)=2.658$	NS	$F(1,32)=87.325$	<0.001
Percent entries	$F(1,66)=26.713$	<0.001	$F(1,66)=15.609$	<0.001	$F(1,66)=16.042$	<0.001	$F(1,33)=27.292$	<0.001	$F(1,33)=0.002$	NS	$F(1,32)=39.469$	<0.001
β-funaltrexamine												
Percent time	$F(1,108)=1.187$	NS	$F(1,108)=96.343$	<0.001	$F(1,108)=0.234$	NS	$F(1,54)=49.282$	<0.001	$F(1,54)=47.132$	<0.001	$F(1,54)=1.383$	NS
Percent entries	$F(1,108)=1.511$	NS	$F(1,108)=17.697$	<0.001	$F(1,108)=0.217$	NS	$F(1,54)=6.155$	<0.025	$F(1,54)=12.647$	<0.001	$F(1,54)=0.234$	NS
Naltrindole												
Percent time	$F(1,105)=0.301$	NS	$F(1,105)=129.314$	<0.001	$F(1,105)=7.209$	<0.01	$F(1,53)=31.170$	<0.001	$F(1,52)=126.507$	<0.001	$F(1,51)=8.018$	<0.01
Percent entries	$F(1,105)=1.798$	NS	$F(1,105)=34.719$	<0.001	$F(1,105)=6.694$	<0.025	$F(1,53)=5.476$	<0.025	$F(1,52)=35.866$	<0.001	$F(1,51)=5.473$	<0.025
Nor-binaltorphimine												
Percent time	$F(1,102)=0.001$	NS	$F(1,102)=52.287$	<0.001	$F(1,102)=2.522$	NS	$F(1,51)=46.832$	<0.001	$F(1,51)=13.612$	<0.001	$F(1,51)=2.772$	NS
Percent entries	$F(1,102)=1.483$	NS	$F(1,102)=6.685$	<0.025	$F(1,102)=4.112$	NS	$F(1,51)=10.860$	<0.01	$F(1,51)=0.152$	NS	$F(1,51)=4.227$	NS

Two-way ANOVA with antagonist and nicotine treatment as between-subjects factors. One-way ANOVA with Bonferroni's correction was applied for further studies. In order to simplify the table, the comparison between VEH–VEH and ANT–VEH is not shown since it was not significant in any case. Percent of time and percent of entries refer to open arms/total. See "Materials and methods" for details

NIC Nicotine, *VEH* vehicle, *ANT* antagonists, *NS* non-significant

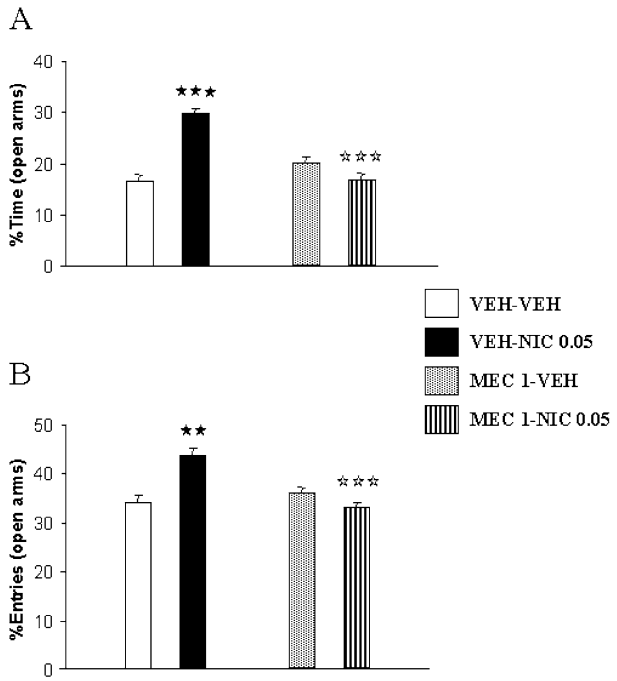

Fig. 1 Mecamylamine (*MEC*) blocks the anxiolytic-like responses induced by nicotine (*NIC*) in the elevated plus maze. **A** Percentage of time spent in the open arms; **B** percentage of entries in the open arms. Mecamylamine (1 mg/kg) and nicotine (0.05 mg/kg) were administered 25 and 5 min before the test, respectively. Data are expressed as mean±SEM (n=18–20 mice for each group). *Two filled starsP*<0.01; *Three filled starsP*<0.001 when comparing with respective vehicle (*VEH*) group; *three open starsP*<0.001 when comparing with nicotine + vehicle group (one-way ANOVA)

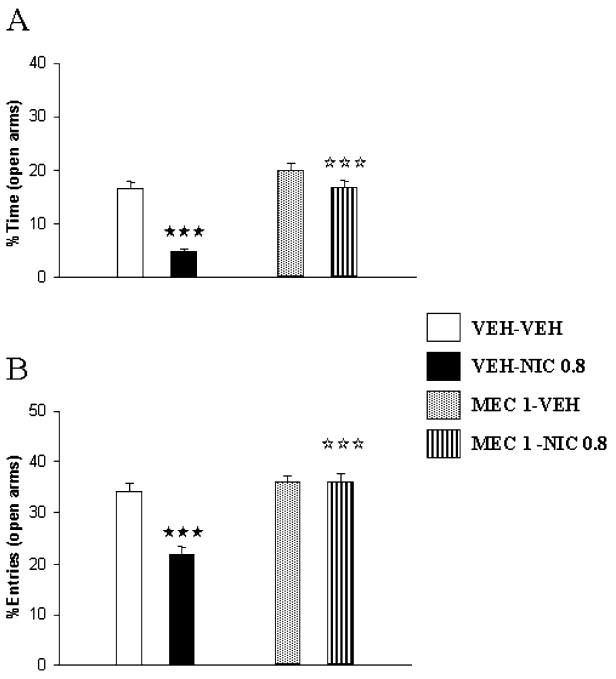

Fig. 2 Mecamylamine (*MEC*) blocks the anxiogenic-like responses induced by nicotine (*NIC*) in the elevated plus maze. **A** Percentage of time spent in the open arms; **B** percentage of entries in the open arms. Mecamylamine (1 mg/kg) and nicotine (0.8 mg/kg) were administered 25 and 5 min before the test, respectively. Data are expressed as mean±SEM (n=17–18 mice for each group). *Three filled starsP*<0.001 when comparing with respective vehicle (*VEH*) group; *three open starsP*<0.001 when comparing with nicotine + vehicle group (one-way ANOVA)

arms. This effect was blocked by β-funaltrexamine (5 mg/kg), which did not induce any response when given alone (Table 2 and Fig. 3A). Two-way ANOVA calculated for the percentage of entries in the open arms showed no effect of nicotine treatment, no effect of antagonist treatment, and a significant interaction between these two factors (Table 2). One-way ANOVA revealed a similar pattern of results to the percentage of time (Table 2 and Fig. 3B).

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

Two-way ANOVA calculated for the percentage of time spent in the open arms showed a significant effect of nicotine treatment, no effect of antagonist treatment, and no significant interaction between these two factors (Table 2). One-way ANOVA revealed that acute nicotine (0.05 mg/kg) alone increased the percentage of time spent in the open arms. A similar increase induced by nicotine was observed in the presence of naltrindole (2.5 mg/kg), which did not produce any response when given alone (Table 2 and Fig. 4A). Two-way ANOVA calculated for the percentage of entries in the open arms revealed a similar pattern of results (Table 2). One-way ANOVA revealed that acute nicotine (0.05 mg/kg) alone increased the percentage of entries in the open arms. However, this significant increase was not observed in the

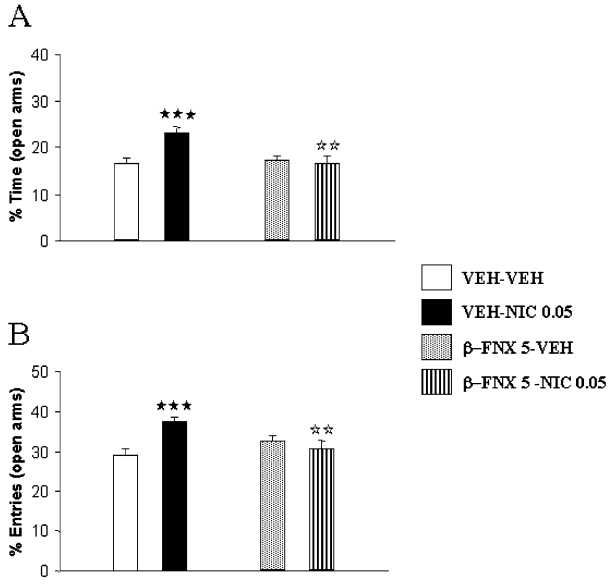

Fig. 3 Effects of pretreatment with β-funaltrexamine (*β-FNX*) on the anxiolytic-like responses induced by nicotine (*NIC*) in the elevated plus maze. **A** Percentage of time spent in the open arms; **B** percentage of entries in the open arms. β-funaltrexamine (5 mg/kg) and nicotine (0.05 mg/kg) were administered 24 h and 5 min before the test, respectively. Data are expressed as mean±SEM (n=27–28 mice for each group). *Three filled starsP*<0.001 when comparing with respective vehicle (*VEH*) group; *two open starsP*<0.01 when comparing with nicotine + vehicle group (one-way ANOVA)

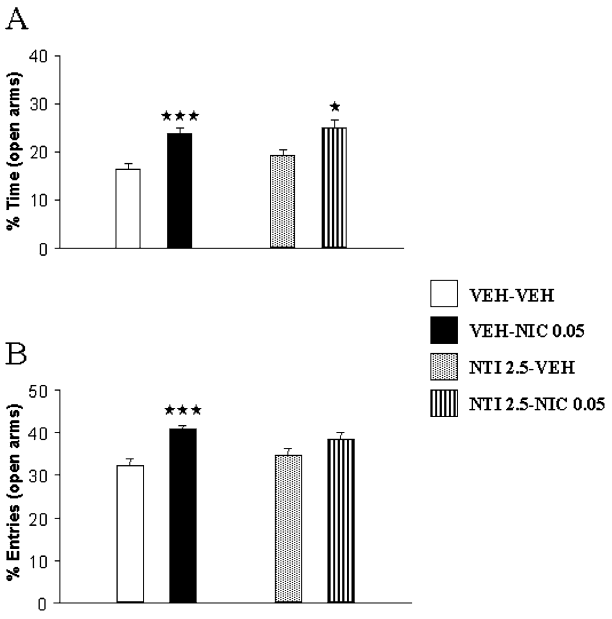

Fig. 4 Effects of pretreatment with naltrindole (*NTI*) on the anxiolytic-like responses induced by nicotine (*NIC*) in the elevated plus maze. **A** Percentage of time spent in the open arms; **B** percentage of entries in the open arms. Naltrindole (2.5 mg/kg) and nicotine (0.05 mg/kg) were administered 25 and 5 min before the test, respectively. Data are expressed as mean±SEM (n=26–28 mice for each group). *One filled star*P<0.05; *three filled stars*P<0.001 when comparing with respective vehicle (*VEH*) group (one-way ANOVA)

presence of naltrindole (2.5 mg/kg), which did not induce any response when given alone (Table 2 and Fig. 4B).

Effects of nor-binaltorphimine on the anxiolytic-like responses induced by nicotine

Two-way ANOVA calculated for the percentage of time spent in the open arms showed a significant effect of nicotine treatment, no effect of antagonist treatment, and no significant interaction between these two factors (Table 2). One-way ANOVA revealed that acute nicotine (0.05 mg/kg) alone increased the percentage of time spent in the open arms. A similar increase induced by nicotine was observed in the presence of nor-binaltorphimine (2.5 mg/kg), which did not produce any response when given alone (Table 2 and Fig. 5A). Two-way ANOVA calculated for the percentage of entries in the open arms revealed a similar pattern of results (Table 2). One-way ANOVA revealed that acute nicotine (0.05 mg/kg) alone increased the percentage of entries in the open arms. This increase was not observed in the presence of nor-binaltorphimine (2.5 mg/kg), which did not induce any response when given alone (Table 2 and Fig. 5B).

Effects of β-funaltrexamine on the anxiogenic-like responses induced by nicotine

Two-way ANOVA calculated for the percentage of time spent in the open arms showed a significant effect of nicotine treatment, no effect of antagonist treatment, and no significant interaction between these two factors (Table 2). One-way ANOVA revealed that acute nicotine (0.8 mg/kg) alone decreased the percentage of time spent in the open arms. A similar decrease induced by nicotine was observed in the presence of β-funaltrexamine (5 mg/kg), which did not produce any response when given alone (Table 2 and Fig. 6A). The same analysis of the percentage of entries in the open arms revealed a similar pattern of results (Table 2 and Fig. 6B).

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

Two-way ANOVA calculated for the percentage of time spent in the open arms showed a significant effect of nicotine treatment, no effect of antagonist treatment, and a significant interaction between these two factors (Table 2). One-way ANOVA revealed that acute nicotine (0.8 mg/kg) alone decreased the percentage of time spent in the open arms. Nicotine also significantly decreased the time spent in the open arms in the presence of naltrindole (2.5 mg/kg), which did not induce any response when given alone (Table 2 and Fig. 7A). Naltrindole pretreatment significantly enhanced the anxiogenic-like responses induced by nicotine. The same analysis of the percentage of entries in the open arms revealed a similar pattern of results (Table 2 and Fig. 7B).

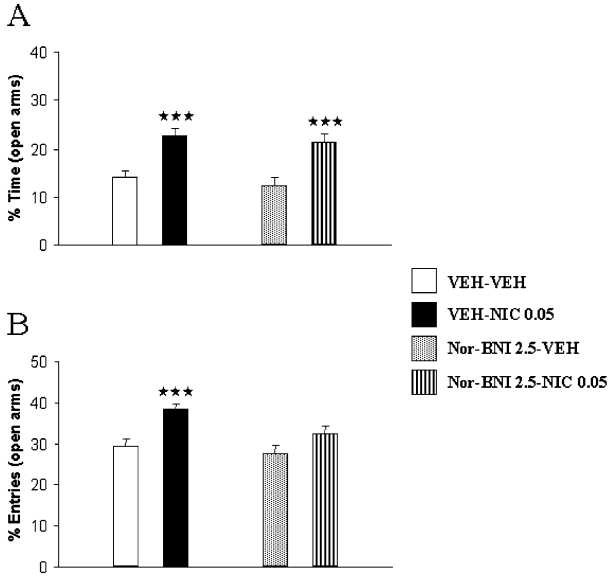

Fig. 5 Effects of pretreatment with nor-binaltorphimine (*nor-BNI*) on the anxiolytic-like responses induced by nicotine (*NIC*) in the elevated plus maze. **A** Percentage of time spent in the open arms; **B** percentage of entries in the open arms. Nor-binaltorphimine (2.5 mg/kg) and nicotine (0.05 mg/kg) were administered 55 and 5 min before the test, respectively. Data are expressed as mean±SEM (n=26–28 mice for each group). *Three filled stars*P<0.001 when comparing with respective vehicle (*VEH*) group (one-way ANOVA)

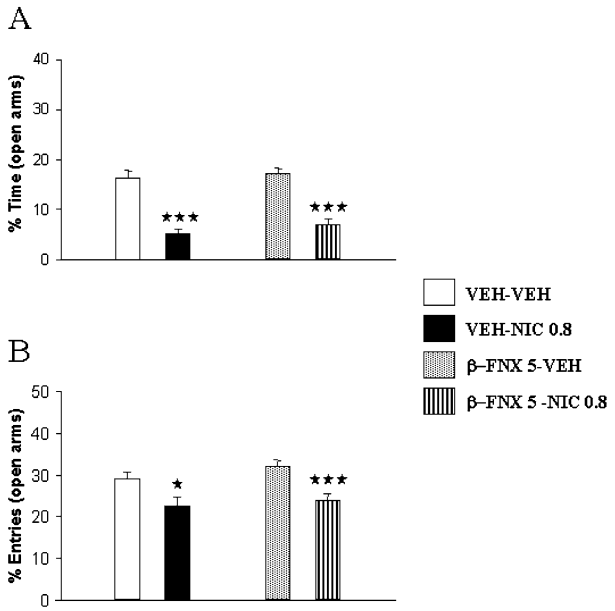

Fig. 6 Effects of pretreatment with β-funaltrexamine (*β-FNX*) on the anxiogenic-like responses induced by nicotine (*NIC*) in the elevated plus maze. **A** Percentage of time spent in the open arms; **B** percentage of entries in the open arms. β-funaltrexamine (5 mg/kg) and nicotine (0.8 mg/kg) were administered 24 h and 5 min before the test, respectively. Data are expressed as mean±SEM (*n*=27–28 mice for each group). *One filled starP*<0.05; *three filled starsP*<0.001 when comparing with respective vehicle (*VEH*) group (one-way ANOVA)

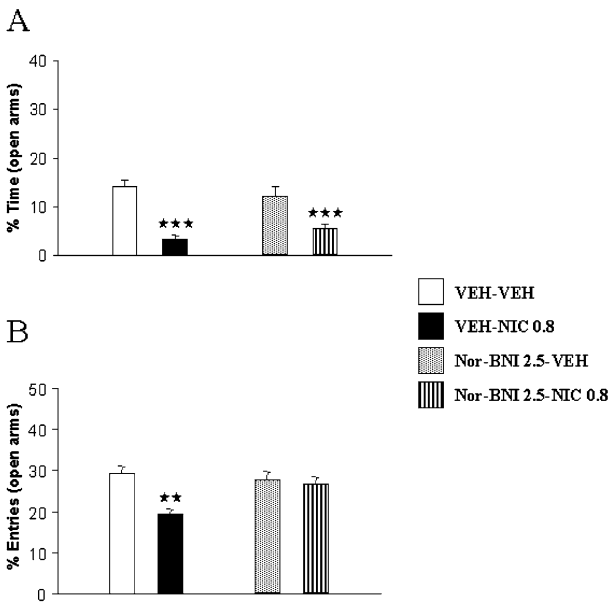

Fig. 8 Effects of pretreatment with nor-binaltorphimine (*nor-BNI*) on the anxiogenic-like responses induced by nicotine in the elevated plus maze. **A** Percentage of time spent in the open arms; **B** percentage of entries in the open arms. Nor-binaltorphimine (2.5 mg/kg) and nicotine (0.8 mg/kg) were administered 55 and 5 min before the test, respectively. Data are expressed as mean±SEM (*n*=26–28 mice for each group). *Two filled starsP*<0.01; *three filled starsP*<0.001 when comparing with respective vehicle (*VEH*) group (one-way ANOVA)

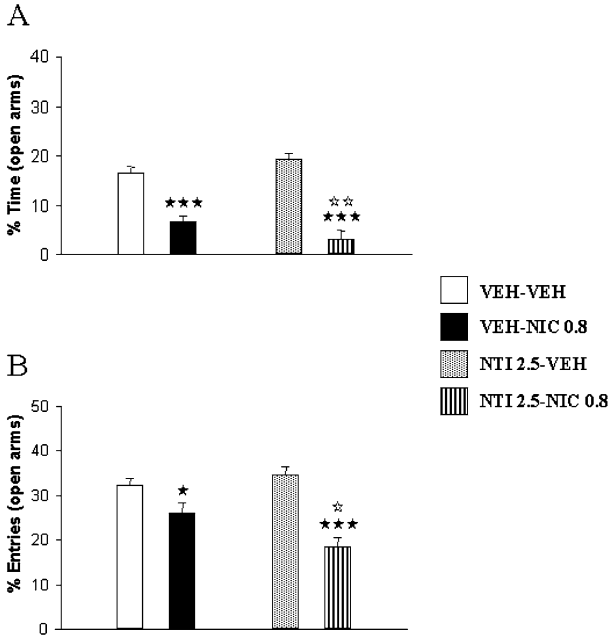

Fig. 7 Effects of pretreatment with naltrindole (*NTI*) on the anxiogenic-like responses induced by nicotine (*NIC*) in the elevated plus maze. **A** Percentage of time spent in the open arms; **B** percentage of entries in the open arms. Naltrindole (2.5 mg/kg) and nicotine (0.8 mg/kg) were administered 25 and 5 min before the test, respectively. Data are expressed as mean±SEM (*n*=26–28 mice for each group). *One filled starP*<0.05; *three filled starsP*<0.001 when comparing with respective vehicle (*VEH*) group; *one open starP*<0.05; *two open starsP*<0.01 when comparing with nicotine + vehicle group (one-way ANOVA)

Effects of nor-binaltorphimine on the anxiogenic-like responses induced by nicotine

Two-way ANOVA calculated for the percentage of time spent in the open arms showed a significant effect of nicotine treatment, no effect of antagonist treatment, and no significant interaction between these two factors (Table 2). One-way ANOVA revealed that acute nicotine (0.8 mg/kg) alone decreased the percentage of time spent in the open arms. A similar decrease induced by nicotine was observed in the presence of nor-binaltorphimine (2.5 mg/kg), which did not produce any response when given alone (Table 2 and Fig. 8A). Two-way ANOVA calculated for the percentage of entries in the open arms showed a similar pattern of results (Table 2). One-way ANOVA revealed that acute nicotine (0.8 mg/kg) alone decreased the percentage of entries in the open arms. This effect was not observed in the presence of nor-binaltorphimine (2.5 mg/kg), which did not induce any response when given alone (Table 2 and Fig. 8B).

Discussion

The present study provides clear evidence for the involvement of the endogenous opioid system in the effects induced by nicotine on anxiety-like responses. Thus, the anxiolytic-like effects induced by nicotine were abolished by the μ-opioid receptor antagonist β-funaltrexamine. In addition, the δ-opioid antagonist, naltrindole, increased

nicotine anxiogenic-like responses, whereas the κ-opioid receptor antagonist, nor-binaltorphimine, failed to significantly modify the effects induced by nicotine on anxiety-like responses.

In the present study, we tested several doses of nicotine (from 0.05 to 0.8 mg/kg) and an anxiolytic-like response was induced by the dose of 0.05 mg/kg of nicotine, whereas an anxiogenic-like effect was only observed after the administration of 0.8 mg/kg of nicotine. These effects elicited by nicotine on anxiety-like behaviour were reversed by the selective nicotinic antagonist, mecamylamine (1 mg/kg), which did not induce any response when given alone at this dose by peripheral route, revealing the direct involvement of nicotinic acetylcholine receptors. However, previous studies have observed that mecamylamine and other cholinergic antagonists have effects on anxiety-like behaviour under different experimental conditions (File et al. 1998; Ouagazzal et al. 1999a,b; File et al. 2000; Newman et al. 2002), and interpretation of results may therefore be difficult. Previous studies have also reported anxiolytic- and anxiogenic-like responses after nicotine administration in rodents (Vale and Green 1986; Costall et al. 1989; Cao et al. 1993; Brioni et al. 1993; File et al. 1998; Ouagazzal et al. 1999a). In agreement with our results, these opposite nicotine effects also depend on the dose in the social interaction test of anxiety in rats, with low doses having an anxiolytic and high doses an anxiogenic action (File et al. 1998). However, factors other than dose, such as strain differences and/or differences in the baseline level of anxiety, could also be important for the effects induced by nicotine in the elevated plus maze in rats (Brioni et al. 1994; Ouagazzal et al. 1999a).

Different brain regions receiving serotonergic projections from forebrain structures have been reported to be involved in these two opposite actions of nicotine, with the dorsal raphe nucleus participating in the anxiolytic-like effects (File et al. 1999; Cheeta et al. 2000a) and the dorsal hippocampus and lateral septum in the anxiogenic-like responses (Ouagazzal et al. 1999b; Cheeta et al. 2000b; Kenny et al. 2000). Nicotine anxiogenic-like effects could be mediated by the enhancement of 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 (Guimaraes et al. 1991; Gray et al. 1994; Jessa et al. 1995; Onaka et al. 1996). The stimulatory effects of nicotine on corticotropin-releasing factor and adrenocorticotropin hormone release (Okuda et al. 1993; Bremner et al. 1996; Herman and Cullinan 1997) could also contribute to these anxiogenic-like responses. On the other hand, the serotonergic system has been reported to participate in the anxiolytic-like effects of nicotine. Thus, the anxiolytic effect induced by local nicotine microinjection into the dorsal raphe nucleus was antagonised by a behaviourally inactive dose of the 5-HT_{1A} receptor antagonist, WAY 100635 (Cheeta et al. 2001).

Another possible neurobiological system implicated in the effects of nicotine on anxiety-like responses is the endogenous opioid system, although its specific involvement has not yet been investigated. Thus, numerous studies have reported a pharmacological interaction between opioids and nicotine, mainly in antinociception, rewarding properties, and physical dependence (Decker and Meyer 1999; Hildebrand et al. 1999; Watkins et al. 2000; Berrendero et al. 2002). The endogenous opioid system has also been suggested to participate in several central effects of nicotine (Balfour 1982). Furthermore, the colocalisation at regional levels of both nicotinic and opioid receptors in several limbic structures, including the nucleus accumbens and the amygdala (Mansour et al. 1995), raises the hypothesis of an interaction between these two systems to control anxiety-like responses. In the present study, we observed that the activity of μ-opioid receptors is necessary for nicotine to induce anxiolytic-like effects since this response was abolished by the μ-opioid antagonist β-funaltrexamine. In line with these data, β-funaltrexamine also attenuated the anti-conflict effects of diazepam (Tsuda et al. 1996) and THC anxiolytic-like effects in the light–dark box (Berrendero and Maldonado 2002). However, mice lacking μ-opioid receptors showed lower levels of anxiety in the elevated plus maze (Filliol et al. 2000), although no significant changes were found in the light–dark box. We observed an increase in the anxiogenic-like effects of nicotine when animals were pretreated with the δ-opioid antagonist, naltrindole. Besides, the δ-opioid receptor knockout mice showed spontaneous anxiogenic-like responses in the elevated plus maze and the light–dark box, suggesting that the basal activity of δ-opioid receptors contributes to decrease the level of anxiety (Filliol et al. 2000). In addition, the δ-opioid antagonist, naltrindole, also abolished THC anxiolytic-like effects in the light–dark box (Berrendero and Maldonado 2002). Our results also showed that the administration of the κ-opioid antagonist nor-binaltorphimine failed to significantly modify nicotine-induced anxiolytic- and anxiogenic-like responses. Accordingly, κ-opioid receptor knockout mice showed no phenotype in the elevated plus maze and the light–dark box (Filliol et al. 2000). The doses of the three opioid antagonists used in the present study were selective enough to rule out a possible cross-reactivity with other opioid receptors. Indeed, previous studies have demonstrated such selectivity even using higher doses of these opioid antagonists. Thus, Ward et al. (1985) have shown a selective blockade of μ-opioid receptor on mouse brain membranes after subcutaneous injection of β-funaltrexamine at the dose of 100 mg/kg, without affecting the binding of [^{3}H]methionine enkephalin. On the other hand, subcutaneous administration of a high dose of naltrindole (20 mg/kg) in mice selectively antagonised the antinociceptive effects induced by a δ-opioid agonist, but did not modify the responses induced by μ- or κ-opioid agonists (Portoghese et al. 1988). Finally, subcutaneous administration of nor-binaltorphimine at the dose of 5 and 20 mg/kg selectively antagonised U-50488H-induced antinociception in the tail pinch test (Endoh et al. 1992).

The mechanisms underlying these nicotinic–opioid interactions have not yet been clarified. One possibility to explain the present findings is that nicotine, through acti-

vation of nicotinic receptors, may increase the release of endogenous opioid peptides in structures involved in the control of emotional responses. Accordingly, studies using Tyr–Gly–Gly, an extraneuronal metabolite of endogenous enkephalins, have suggested that acute administration of nicotine induces enkephalin release in several brain regions including the nucleus accumbens, without affecting brain enkephalin level (Houdi et al. 1991). Other studies have also suggested that acute nicotine administration induces the release of endogenous opioid peptides in several brain areas in rodents related to the rewarding effects of nicotine (Pierzchala et al. 1987; Dhatt et al. 1995). In humans, cigarette consumption increases plasma levels of beta-endorphin (Pomerleau et al. 1983), and endogenous opioids seem to participate in the reinforcement of smoking since the opioid antagonists naloxone and naltrexone modulate cigarette consumption and subjective pleasure derived from smoking (Karras and Kane 1980; Wewers et al. 1998).

In conclusion, we provide the first pharmacological evidence to reveal the specific involvement of μ-, δ-opioid receptors in mediating the effects induced by nicotine on anxiety-like behaviour. Thus, the blockade of μ-opioid receptor abolished the anxiolytic-like responses of nicotine. δ-Opioid receptor antagonism significantly increased the anxiogenic-like response of nicotine, whereas the blockade of κ-opioid receptor failed to modify these responses. The elucidation of this new interaction between nicotine and the endogenous opioid system provides a further step for better understanding the complex behavioural responses of this drug. Changes induced by nicotine on human emotional responses are indeed important for the instatement and maintenance of tobacco consumption and addiction.

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