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Acute effects of nicotine on restraint stress-induced anxiety-like behavior, c-Fos expression, and corticosterone release in mice

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Abstract

Clinical evidence suggests that nicotine reduces anxiety in stressful situations. In the present study, we investigated the effect of nicotine on restraint-enhanced anxiety-like behavior, c-Fos expression, an index of neuronal activation in the brain, and plasma corticosterone. Two-hour restraint stress-enhanced anxiety-like behavior in the elevated plus-maze (EPM) and nicotine hydrogen tartrate (0.25 mg/kg, i.p.) attenuated the stress-induced changes. Pretreatment with the centrally acting nicotinic antagonist, mecamylamine (2 mg/kg), blocked nicotine's effects. In addition, restraint led to significant increases of c-Fos expression in several brain regions related to stress or anxiety including paraventricular hypothalamic nucleus (PVN), lateral hypothalamic area (LH), central amygdaloid nucleus (CeA), medial amygdaloid nucleus (MeA) and cingulate and retrosplenial cortices (Cg/RS), paraventricular thalamic nucleus (PVT), and basolateral amygdaloid nucleus (BLA). Nicotine attenuated the restraint-induced expression of c-Fos in the PVN, LH, CeA, MeA, and Cg/RS, while leaving the BLA and PVT unaffected. In contrast, nicotine did not reverse the increased levels of plasma corticosterone induced by restraint. These findings suggest that nicotine may modify the stress-induced behavioral changes via regulating the neuronal activation in selected brain regions rather than affecting hypothalamo-pituitary-adrenocortical axis hormone responses.

Keywords: Nicotine; Stress; Anxiety; c-Fos; Corticosterone

1. Introduction

In spite of heightened education and prevention strategies, cigarette smoking remains a major health risk. Nicotine is believed to be the primary reason that people consume tobacco products. It is of interest to understand the behavioral effects of nicotine that might contribute to ongoing tobacco use. It has been shown that cigarette smoking can reduce anxiety and relieve stress in humans, which are likely attributable to the nicotine contained in cigarette (Kassel and Unrod, 2000; Pomerleau et al., 1984). Numerous human studies have demonstrated that an increase in smoking among smokers when exposed to stress, and such behavior is believed to reduce the subjective feeling of stress-related tension (Parrott, 1994; Todd, 2004). However, nicotine dependency is associated with mood lability, leading to heightened feelings of stress in many regular smokers and stress level has been shown to decline after smoking cessation (Parrott, 2004). Actually, smoking under stress bring smokers to stress levels that are comparable to non-smokers not smoking, suggesting that acute nicotine deprivation (i.e., between cigarettes) leads to increased stress, and nicotine reinstatement relieves feelings of stress (Parrott, 2003). It still remains controversial whether nicotine actually reduces stress.

Anxiety is thought to be a negative emotion caused by many kinds of stress. In the field of anxiety, the elevated plus-maze (EPM) has become one of the most popular animal models. The anxiety-like behavior (i.e., decreased percentage time spent in the open arms) is potentiated in the EPM by prior exposure to a variety of stressors, such as restraint, social defeat, forced swim, and inescapable footshock (Mechiel Korte and De Boer, 2003) Nicotine has been shown to affect anxiety in different ways in animal studies. In rodents, nicotine can be anxiolytic, anxiogenic, or have no effect on anxiety, depending on the dose

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used, the route of administration, and the time of testing after nicotine administration even when the same behavioral test is performed (Brioni et al., 1993; Cheeta et al., 2001b; Costall et al., 1989; Dayas et al., 2001a; Ouagazzal et al., 1999; Szyndler et al., 2001; Vale and Green, 1996). However, there is no report related to the effects of nicotine on stress-enhanced anxiety.

Many studies have explored the central pathways mediating the stress response by mapping neuronal activation using the proto-oncogene c-*fos* (Arnold et al., 1992; Chen and Herbert, 1995). Although differential patterns of neuronal activation induced by stress are reported in different studies, in terms of the nature of the stress (Dayas et al., 2001b; Emmert and Herman, 1999) and the intensity of the stimulus (Campeau and Watson, 1997), certain forebrain regions are consistently implicated in gating the stress-induced inputs to the paraventricular nucleus of the hypothalamus (PVN). Medial prefrontal cortex, central amygdale (CeA), bed nucleus of the stria terminalis (BNST), and hippocampus, comprising major regulatory inputs to the PVN, have been implicated in the production of the enhanced anxiety state induced by stress (Dazzi et al., 2001; Figueiredo et al., 2003; Hutson and Barton, 1997; Jackson and Moghaddam, 2006; Mechiel Korte and De Boer, 2003) or anxiogenic drugs (Singewald et al., 2003). The purpose of the present study was to investigate how the impact of nicotine might modify the effects of previous exposure to a restraint stress on the EPM test of anxiety, restraint-induced neuronal activation as measured by c-Fos expression in the brain regions recognized important to stress and/or anxiety, and restraint-enhanced serum corticosterone.

2. Materials and methods

2.1. Animal treatment

Male NMRI mice (8–9 weeks, 33–40 g) were supplied from the Laboratory Animal Center of Tzu Chi University (Hualien, Taiwan). They were housed in groups of five under conditions of controlled temperature (22±1 °C) and lighting (lights on from 7 AM to 7 PM); food pellets and water were freely available. The experimental protocol was approved by the Review Committee of the Tzu Chi University for the Use of Animal.

2.2. Chemicals

The drugs used, (−) nicotine hydrogen tartrate and mecamylamine (from Sigma), were dissolved in saline and injected intraperitoneally in a volume of 0.1 ml/10 g body weight. All injection solutions were freshly prepared daily, and the pH of the saline and nicotine solutions was adjusted to pH 7.4 with sodium hydroxide.

2.3. Experimental design

In the preliminary experiment, mice were restraint stressed for various time periods (30, 60, and 120 min) and then returned to their home cage for 30 min before behavioral testing. Restraint for 120 min was applied for all the other experiments. Nicotine has been reported to produce anxiolytic- and anxiogenic-like responses. In order to determine the dose of nicotine, which can produce an anxiolytic response, behavioral responses to various dose (0.05–0.5 mg/kg; dose given as salt) of nicotine in the EPM were examined 30 min and 150 min after nicotine treatment. The dose of nicotine chosen for the following experiments was 0.25 mg/kg (0.0875 mg/kg calculated as the base).

2.3.1. Experiment I. Effect of nicotine on restraint-enhanced anxiety behavior in the EPM

Animals that had been assigned to the restraint groups were removed from their home cage and individually placed in a round plastic container (3 cm in diameter, 12 cm in length). In order to clarify if nicotine exerts its effects during or after the restraint stress, nicotine (0.25 mg/kg) and vehicle were administered immediately prior to or after a 120-min restraint stress. EPM was tested 30 min after restraint.

2.3.2. Experiment II. Effect of mecamylamine on the nicotine-induced lower anxiety-like behavior in the EPM after restraint stress

Animals were distributed to 5 groups: Saline–Saline-Control; Saline–Saline-Restraint; Saline–Nicotine-Restraint; Mecamylamine–Saline-Restraint; Mecamylamine–Nicotine-Restraint. Mecamylamine (2 mg/kg) was administered 10 min before nicotine (0.25 mg/kg) and restraint stress. Behavioral test was examined 30 min after restraint.

2.3.3. Experiment III. Effect of nicotine on restraint-induced c-Fos expression and corticosterone release

Animals were distributed to 4 groups: Saline-Control; Saline-Restraint; Nicotine-Control; Nicotine-Restraint. Thirty minutes after restraint, the animals were sacrificed.

2.4. Elevated plus-maze

The plus-maze was constructed of Plexiglas and consisted of two open and two closed arms (10 cm wide × 50 cm long, 50 cm walls for closed, 2 cm walls for open), intersected by a center platform (100 cm^2), elevated 50 cm off the floor. Each animal was tested for 5 min on the maze and videotaped. A mouse was placed on the central platform of the maze facing the open arm. The following indices were recorded: the total number of entries into open arm and closed arm and the total time spent in each type of arm. From these values, the percentage of time spent in the open arms provided as the measures of anxiety was calculated for each animal. The number of closed arm entries provides the measure of locomotor activity in this test. An entry was defined as the entry of all four feet into one arm and an arm exit was defined as two paws leaving the arm. Between tests, the maze was wiped clean.

2.5. Immunohistochemistry

Thirty minutes after restraint, mice were deeply anesthetized with sodium phenobarbital (65 mg/kg, i.p.) and then perfused

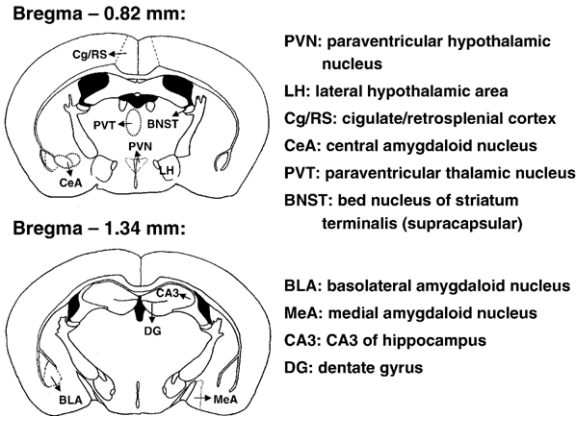

Fig. 1. Schematic diagrams of analyzed brain region adapted from the mouse brain atlas (Paxinos and Franklin, 1997).

transcardially with 300 ml of heparin in 0.1 M phosphate-buffered saline (PBS), subsequently with cold fixative (4% paraformaldehyde in 0.1 M PBS, pH 7.4). Brains were dissected, postfixed for 16 h and then cryoprotected with 30% sucrose solution at least until it sank. The brain was frozen and cut into 20 μm think coronal sections in a cryostat. Free-floating sections were processed for c-Fos immunohistochemistry. Sections were incubated for 30 min in 1% H_2O_2 to inactivate endogenous peroxidase activity and decreased non-specific staining. Sections were washed three times (10 min per wash) in 0.01 M PBS containing 0.4% Triton X-100 and 2% BSA. Blocking was performed with 10% BSA for 1 h and then incubated on rocking table with c-Fos polyclonal antibody (Santa Cruz, 1:5000) for overnight at 4 °C. The sections were washed again three times in PBS prior to being incubated with the biotinylated goat anti-rabbit secondary antibodies for 1.5 h at room temperature. After again washing three times in PBS buffer, the sections were incubated with the 0.2% avidin-biotinylated horseradish peroxidase complex (Vector Laboratories) for 1 h. After three 10-min washes in PBS, the sections were placed in the chromogen 3,3′-diaminobenzidine tetrahydrochloride (DAB, Sigma, St. Louis, MO) for 3 min. The sections were again washed three times in PBS, mounted on gelatinized slides and left overnight to dry. The mounted sections were dehydrated through a graded alcohol series to xylene and were coverslipped. No immunoreactivity was observed when the primary antibody was omitted.

c-Fos immunoreactivity was observed in the cell nuclei and brain sections were shown as a brownish round dot under a light microscope. Coronal brain slices at the levels of Bregma −0.82 mm and −1.34 mm (Paxinos and Franklin, 1997) were selected for cingulate/retrosplenial cortex (Cg/RS), paraventricular nucleus of the hypothalamus (PVN), paraventricular thalamic nucleus (PVT), bed nucleus of striatum terminalis (BNST), central amygdale (CeA), as well as medial amygdaloid nucleus (MeA), basal lateral amygdala (BLA) and dentate gyrus, CA3 of hippocampus, respectively (Fig. 1).

Quantification of Fos positive nuclei were counted with the aid of a Nikon Eclipse 800 microscope, equipped with a Polaroid DMC digital camera (1600×1200 dpi in 8 bits) with 100× magnification, using the Image Pro Plus 4.5 morphometry program (Media Cybernetics, Silver, MD). Following background subtraction, the threshold was adjusted so that pale- and

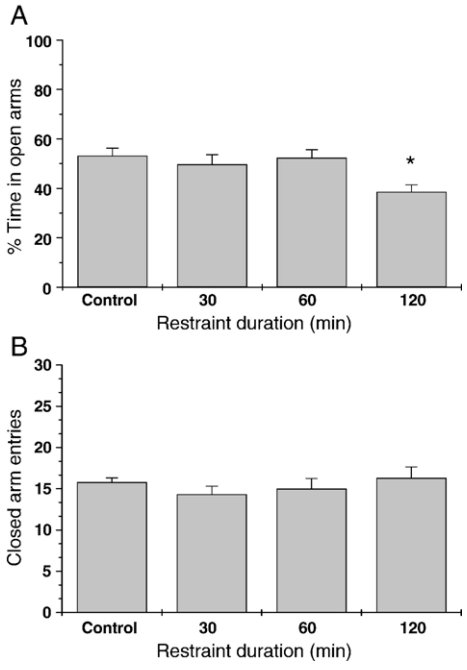

Fig. 2. Effects of restraint duration on anxiety-like behavior in the EPM. The mice were subjected to the EPM 30 min following restraint for 0, 30, 60, and 120 min. The level of anxiety, as judged by the decrease in the percentage time spent in the open arms (A) and the number of closed arm entries (B) were recorded. Values are mean ± S.E.M. (n=8).* P<0.05 vs. control group.

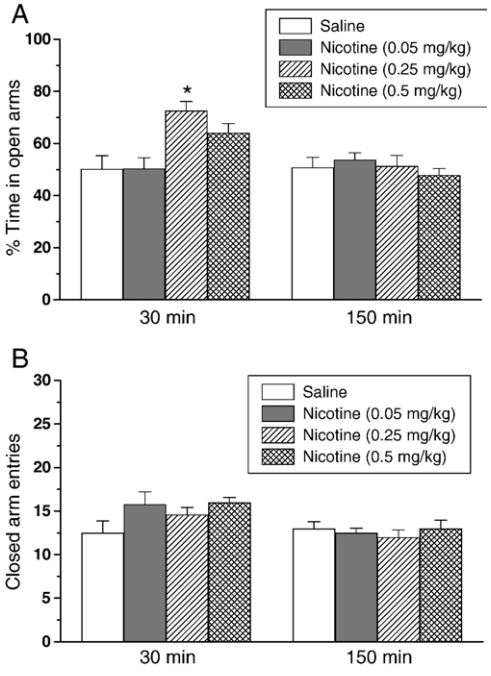

Fig. 3. Dose- and time-dependent effects of nicotine on anxiety-like behavior in the EPM. Various doses of nicotine (0–0.5 mg/kg) were administered 30 or 150 min prior to EPM test. The percentage time spent in the open arms (A) and the number of closed arm entries (B) in the EPM were recorded. Values are mean ± S.E.M. (n=8).* P<0.05 vs. Saline group.

deep-stained nuclei could be equally recognized by the counting program. The area of interest was the rectangular image-capturing field of the camera. The square area of this field is 0.16 mm^2. Cell counts were made with the help of the Image Pro Plus 4.5 software and manually counted by an observer who was blind to the group assignment. The value in each area was obtained from means of both the right and left sides of four subjects, in each treatment group.

2.6. Corticosterone measurement

Trunk blood was collected in tubes after decapitation. To obtain plasma, samples were centrifuged at 1000 ×*g* for 15 min. Corticosterone EIA kit (Cayman Chemical Co.) was used for the quantitative determination of plasma corticosterone. The detailed procedure was according to the manufacturer's instruction.

2.7. Statistical analysis

Values represent the mean±S.E.M. for groups. Data were analyzed by one-way or two-way ANOVA followed by Newman–Keuls multiple comparison test.

3. Results

In the preliminary experiments, restraint stress for 120 min, but not 30 and 60 min, reduced the percentage of time spent in the open arms in the EPM ($F_{3,28}$=4.204, P=0.014) (Fig. 2A).

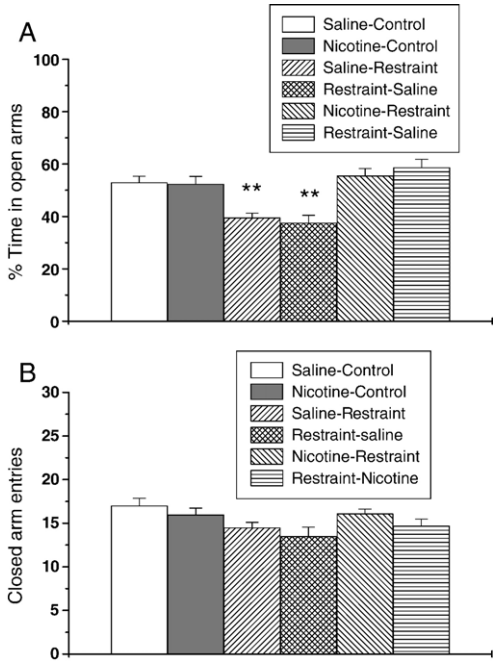

Fig. 4. Effect of nicotine on restraint-enhanced anxiety-like behavior in the EPM. Mice were administered with nicotine (0.25 mg/kg) or saline before or after the 120-min restraint stress and then subjected to EPM 30 min after restraint. The percentage time spent in the open arms (A) and the number of closed arm entries (B) in the EPM were recorded. Values are mean ± S.E.M. (n=10).** P<0.01 vs. Saline-Control group.

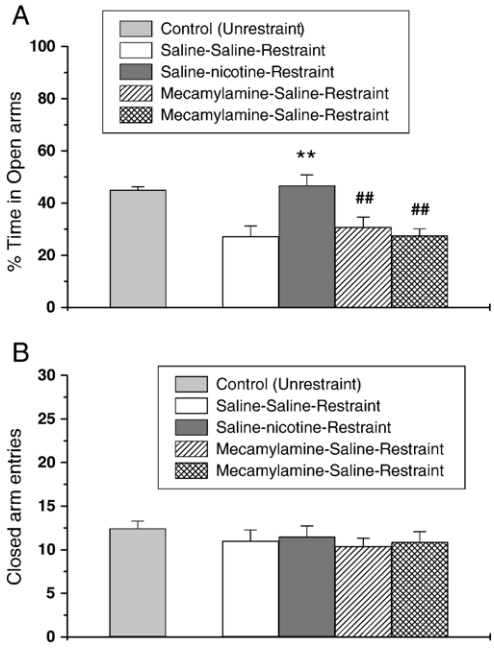

Fig. 5. Mecamylamine reverses the effect of nicotine on restraint-enhanced anxiety-like behavior in the EPM. Mice pretreated with mecamylamine (2 mg/kg) or saline were administered with nicotine (0.25 mg/kg) or saline followed by 120-min restraint stress and then subjected to EPM. The percentage time spent in the open arms (A) and the number of closed arm entries (B) in the EPM were recorded. Values are mean ± S.E.M. (n=8).** P<0.01 vs. Saline-Saline-Control group.

The activity, measured as number of closed arm entries was not affected by restraint duration ($F_{3,28}$=0.66, P=0.58) (Fig. 2B) The animals received 0.25 mg/kg of nicotine 30 min ($F_{3,28}$=7.53, P=0.008), but not 150 min ($F_{3,28}$=0.42, P=0.74) before EPM test displayed lower anxiety-like behavior (Fig. 3A) without any effects on the number of closed arm entries ($F_{3,28}$=1.77, P=0.17) (Fig. 3B). Therefore, nicotine (0.25 mg/kg) and 120-min restraint were applied for the following experiments.

Table 1
Interactions between nicotine and restraint stress in the c-Fos expression

Region	Control		Restraint	
	Saline	Nicotine	Saline	Nicotine
PVN	10.41±2.04	12.58±3.78	80.08±17.76[a]	32.50±10.97[c]
LH	0.16±0.09	0.08±0.08	45.33±5.24[b]	10.91±4.94[e]
Cg/RS	0.41±0.31	0.67±0.66	12.08±1.79[b]	5.87±1.96[d]
CeA	0.16±0.16	0±0	9.50±2.06[b]	4.58±1.14[c]
MeA	3.91±1.67	7.83±4.83	20.58±5.26[a]	8±1.9[c]
BLA	1±0.49	0.25±0.15	5.20±1.32[a]	4.50±0.79
PVT	15.33±3.17	16.50±2.74	50.33±7.38[b]	48.66±4.99
CA3	1±0.49	0.66±0.66	3.79±1.11	5.75±1.42
DG	2.45±0.20	2.66±0.19	2.25±0.47	3.41±1.31
BNST	0.41±0.41	0±0	0±0	0.16±0.09

Values represent the mean number of cells (± S.E.M.) per tissue area (0.16 mm^2) in the paraventricular hypothalamic nucleus (PVN), lateral hypothalamic area (LH), central amygdaloid nucleus (CeA), medial amygdaloid nucleus (MeA) and cingulate and retrosplenial cortices (Cg/RS), paraventricular thalamic nucleus (PVT), and basolateral amygdaloid nucleus (BLA). CA3 subfield of hippocampus (CA3), dentate gyrus (DG), and bed nucleus of the stria terminalis (BNST). [a]P<0.01, [b]P<0.001 vs. Saline-Control group. [c]P<0.05, [d]P<0.01, [e]P<0.001 vs. Saline-Restraint group (n=4).

Table 2
P values from the two-way ANOVA of the effects of nicotine and restraint on c-Fos expression

	Restraint	Nicotine	Interaction
PVN	0.001	0.055	0.038
	$F_{(1,12)}=17.65$	$F_{(1,12)}=4.54$	$F_{(1,12)}=5.45$
LH	<0.001	<0.001	<0.001
	$F_{(1,12)}=60.41$	$F_{(1,12)}=22.928$	$F_{(1,12)}=22.707$
Cg/RS	<0.001	0.052	0.037
	$F_{(1,12)}=37.36$	$F_{(1,12)}=4.658$	$F_{(1,12)}=5.472$
CeA	<0.001	0.036	0.048
	$F_{(1,12)}=39.93$	$F_{(1,12)}=5.56$	$F_{(1,12)}=4.87$
MeA	0.044	0.265	0.048
	$F_{(1,12)}=5.06$	$F_{(1,12)}=1.365$	$F_{(1,12)}=4.872$
BLA	<0.001	0.39	0.98
	$F_{(1,12)}=26.788$	$F_{(1,12)}=0.796$	$F_{(1,12)}=0.00065$
PVT	<0.001	0.957	0.761
	$F_{(1,12)}=54.67$	$F_{(1,12)}=0.00303$	$F_{(1,12)}=0.0973$
CA3	0.003	0.455	0.298
	$F_{(1,12)}=54.67$	$F_{(1,12)}=0.6$	$F_{(1,12)}=1.193$
DG	0.711	0.355	0.515
	$F_{(1,12)}=0.144$	$F_{(1,12)}=0.927$	$F_{(1,12)}=0.45$
BNST	0.568	0.568	0.198
	$F_{(1,12)}=0.345$	$F_{(1,12)}=0.345$	$F_{(1,12)}=1.855$

Values shown are derived from two-way ANOVA.

3.1. Effect of nicotine on restraint-enhanced anxiety-like behavior in the EPM

Nicotine was administered before and after restraint to determine its effect on restraint-enhanced anxiety-like behavior. Two factors, nicotine and restraint stress (stress first or stress second), were analyzed by a two-way ANOVA. The results showed significant differences in the effect of nicotine ($F_{1,54}=31.65$, $P<0.001$), but not restraint ($F_{2,54}=31.65$, $P=0.115$), on the percentage of time spent in the open arms. However, nicotine and restraint produced significant interaction ($F_{2,54}=9.11$, $P<0.001$) (Fig. 4). Post hoc test indicated that either restraint stress before or after saline injection significantly reduced the percentage of time spent in the open arms ($P<0.001$) and nicotine (0.25 mg/kg) administration either before or after the 120-min restraint stress could reverse the restraint-induced decreased percentage of time spent in the open arms ($P<0.001$) (Fig. 4).

3.2. Effect of blockade of nicotinic receptor on nicotine's suppression of restraint-induced anxiety-like behavior in the EPM

The non-selective nicotinic acetylcholine receptor antagonist mecamylamine (Fig. 5) was used to block the effects of nicotine on restraint-induced anxiety-like behavior. Two-way ANOVA did not show significant effects for treatment of mecamylamine ($F_{1,28}=2.63$, $P=0.116$) and nicotine ($F_{1,28}=2.887$, $P=0.1$). However, there was a significant interaction between mecamylamine and nicotine ($F_{1,28}=6.3$, $P=0.018$). Post hoc tests indicated that nicotine significantly increased the percentage of time spent in the open arms in saline groups ($P=0.006$) and pretreatment with mecamylamine reversed nicotine's effect ($P=0.007$).

3.3. Effect of nicotine on restraint-induced c-Fos expression

According to the previous data, nicotine could not affect the anxiety-like behavior in the EPM when nicotine was

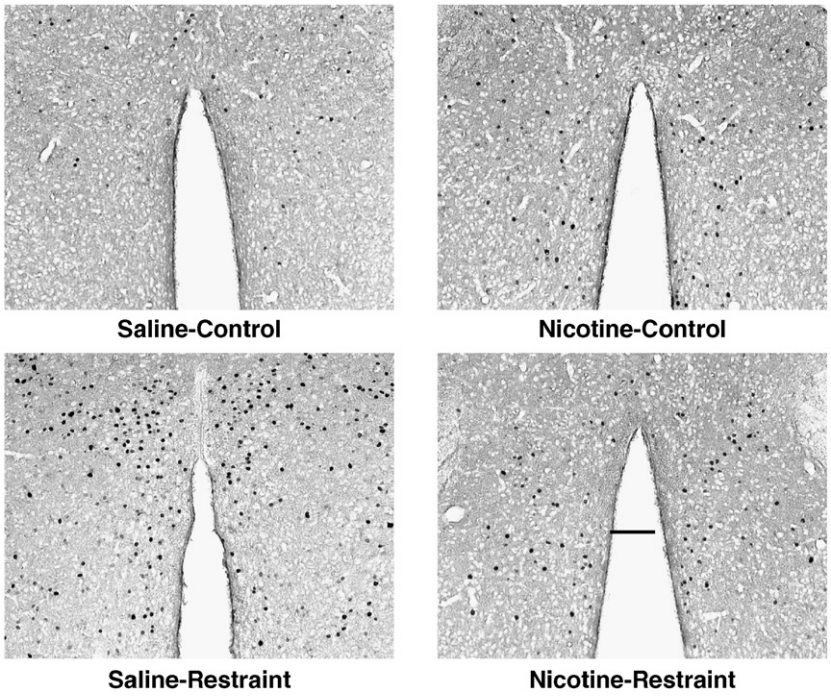

Fig. 6. Representative brightfield photomicrographs showing the interactions between restraint stress and nicotine in the expression of c-Fos protein within the paraventricular hypothalamic nucleus (PVN). Note the robust c-Fos expression in the Saline-Restraint group only. Scale bar=100 μm.

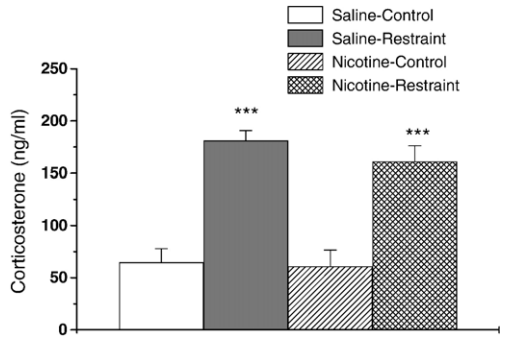

Fig. 7. Effect of nicotine on restraint-induced corticosterone release. Values are mean ± S.E.M. (n=4).*** P<0.001 vs. Saline-Control group.

administered 150 min prior to EPM test, but nicotine could reverse the restraint-enhanced anxiety-like behavior when nicotine was administered 150 min prior to EPM test with 120-min restraint immediately following drug treatment. In order to determine whether nicotine has the ability to reduce the restraint-induced neuronal activation, the quantitative analysis of c-Fos immunoreactive neurons was performed. As shown in Tables 1 and 2, restraint for 120 min induced significant c-Fos expression in the PVN, LH, Cg/RS, CeA, MeA, PVT, and BLA. Nicotine at this dose (0.25 mg/kg) did not produce c-Fos expression, but significantly reduced the restraint-induced c-Fos expression in PVN (Fig. 6), LH, Cg/RS, CeA, and MeA but not PVT and BLA.

3.4. Effect of nicotine on increased corticosterone level by restraint

To investigate whether the ameliorating effects of nicotine on restraint-enhanced anxiety-like behavior could be explained by reduced the activation of hypothalamic–pituitary–adrenocortical axis, the concentrations of plasma corticosterone were compared (Fig. 7). Two-way ANOVA analysis indicated that restraint significantly increased the levels of plasma corticosterone ($F_{1,16}$=65.99, P<0.001). However, nicotine administration did not affect the levels of plasma corticosterone ($F_{1,16}$= 0.81, P=0.38). There is no interaction between restraint and nicotine treatment ($F_{1,16}$=0.37, P=0.55).

4. Discussion

We have demonstrated that acute nicotine (0.25 mg/kg, i.p.) produced an anxiolytic effect in the EPM 30 min, but not 150 min later in mice. In addition, nicotine reduced the restraint-enhanced anxiety-like behaviors and also the restraint-induced c-Fos expression in several brain regions implicated in stress and anxiety. However, the restraint-induced increase in plasma corticosterone was not affected by nicotine. In rats, acute systemic nicotine has been reported to be anxiolytic (Brioni et al., 1993), anxogenic (Cheeta et al., 2001a; Ouagazzal et al., 1999), or to have no effect (Balfour et al., 1986) in the EPM. We found that nicotine exerted an anxiolytic effect at the dose of 0.25 mg/kg in mice. In addition, it has been shown that rats submitted to 2 h of restraint display a reduction of open arm exploration in an EPM immediately after release and still present 24 h later (McBlane and Handley, 1994; Mendonca Netto and Guimaraes, 1996; Padovan and Guimaraes, 2000). Our data from mice demonstrated 2 h of restraint enhanced the anxiety-like behavior in the EPM 30 min later.

In general, stress can produce an array of endocrine, autonomic and behavioral responses. Nicotine has been shown to attenuate stress-induced decrease in locomotor activity (Minowa et al., 2000) and plasma serotonergic measurements (Takada et al., 1995). In the present study, nicotine reversed the restraint-induced anxiety-like behaviors and neural excitatory input to certain anxiety-related brain regions (represented primarily by c-Fos expression). However, there was no effect of nicotine on restraint-induced increase in plasma levels of corticosterone. Actually, nicotine dose-dependently increases the levels of plasma corticosterone soon after drug administration and the levels of plasma corticosterone have returned to control values by 120 min (Porcu et al., 2003). Furthermore, it has been reported that nicotine administration during restraint stress enhances the increase in plasma corticosterone, as compared to the responses induced by either factor alone (Morse, 1989). In our experiment condition, the blood samples were collected 150 min after nicotine injection, thus the levels of plasma corticosterone were not altered by nicotine, but the remarkable effects of restraint still exist. The disparity of nicotine's effect on stress-induced responses indicates that stress-induced anxiety-like behavior might be dissociated with hypothalamic–pituitary–adrenocortical system axis activation. Consistent with our results, it has been reported that behavioral responses to stress are intact, but the stress-related activation of hypothalamic–pituitary–adrenocortical system is absent in CRF-deficient mice (Dunn and Swiergiel, 1999). Similarly, the selective CRF_2 receptor antagonist antisauvagine-30 reduces anxiety-like behavior, but not changes the ACTH response to restraint stress (Pelleymounter et al., 2002). According to the dissociation of anxiety-like behavior with hypothalamic–pituitary–adrenocortical axis activation, it is suggested that nicotine might selectively modify the neuronal circuits related to behavioral outcomes rather than hormonal response to stress.

Many studies have explored the specific circuitry of the brain mediating the stress response by mapping neuronal activation using the proto-oncogene c-*fos* (Crane et al., 2005; Cullinan et al., 1995; Dayas et al., 2001a; Windle et al., 2004) and indicated that widespread regions of the brain are activated by restraint stress. In our study, a significant elevation of c-Fos expression was observed in several brain regions involved in coordinating fear and anxiety responses including PVN, LH, CeA, MeA Cg/RS, PVT, and BLA. Nicotine significantly reduced, but not totally blocked the elevation of c-Fos expression induced by restraint in PVN, LH, CeA, MeA, Cg/RS, while leaving PVT, and BLA unaffected. It is well known that activation of the PVN promotes the release of a variety of hormones that are involved in the neuroendocrine and autonomic responses to fear and psychological stress (Van de Kar and Blair, 1999), whereas stimulation of the lateral hypothalamus seems to be important for cardiovascular expressions of fear and anxiety (Fendt and Fanselow, 1999). Parts of the prefrontal cortex,

including the cingulate cortex, have been reported to influence induction of anxiety and fear-related behavior and may play a role in modulating autonomic reactions during stress and anxiety (Figueiredo et al., 2003; Shah and Treit, 2003). The central nucleus of the amygdala is seen as the central point for dissemination of anxiety- and fear-related information via its efferent projections into coordinated autonomic, endocrine, and behavioral responses (Charney et al., 1998; Gorman et al., 2000). It is noteworthy although the PVN is essential for regulation of the hypothalamic–pituitary–adrenocortical axis, our data showed that nicotine reduced the stress-elicited c-Fos expression in the PVN without affecting the hypothalamic–pituitary–adrenocortical axis hormone secretion (corticosterone). Conversely, it has been reported that acute glucocorticoid pretreatment suppresses stress-induced hypothalamic–pituitary–adrenocortical axis hormone secretion but does not affect c-Fos expression in the PVN (Ginsberg et al., 2003). It appears that stress-induced hypothalamic–pituitary–adrenocortical axis hormone secretion and c-Fos expression in the PVN are not similarly regulated.

In conclusion, the present study is the first to demonstrate that nicotine at least in a specific dose can reduce anxiety-like behavior under restraint stress. This effect, which is independent on the plasma levels of corticosterone, may be associated with the reduction of stress-induced c-Fos expression in certain brain regions involved in stress or anxiety. Therefore, nicotine might modulate the stress-enhanced anxiety via reducing the neuronal activation in anxiety-related brain regions rather than affecting hypothalamic–pituitary–adrenocortical axis hormone responses. These findings support that nicotine plays a role in the tension-reducing effects of smoking.

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