Original Paper

Nicotine-induced anxiogenic-like behaviours of rats in the elevated plus-maze: possible role of NMDA receptors of the central amygdala

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Abstract

The objective of the present study was to investigate the possible role of the *N*-methyl-D-aspartate (NMDA) receptor system of the central amygdala (CeA) in the anxiogenic-like effect of nicotine. Male Wistar rats with cannulas aimed to the CeA were submitted to the elevated plus-maze (EPM). Intraperitoneal (i.p.) injections of nicotine (0.6 and 0.8 mg/kg) decreased percentage open arm time spent (%OAT) and percentage open arm entries (%OAE), but not locomotor activity, indicating an anxiogenic-like response. Bilateral intra-CeA microinjection of NMDA (0.005–0.1 μg/rat) decreased %OAT, but not %OAE and locomotor activity. Moreover, intra-CeA microinjection of NMDA (0.05 μg) with an ineffective dose of nicotine (0.4 mg/kg, i.p.) reduced %OAT and %OAE without effect on locomotor activity. On the other hand, intra-CeA microinjection of the NMDA receptor antagonist D-AP5 (0.05–0.5 μg/rat) increased both %OAT and %OAE, showing an anxiolytic-like effect of the drug. Co-administration of the same doses of D-AP5 with nicotine (0.6 mg/kg, i.p.) increased %OAT and %OAE, but not locomotor activity. Intra-CeA microinjection of D-AP5 reversed the response induced by NMDA (0.1 μg/rat) in the EPM. The results may support the possible involvement of glutamate transmission, through NMDA receptors of central amygdala in the anxiogenic-like effect of nicotine in the EPM task.

Keywords

Nicotine, D-AP5, NMDA, elevated plus maze, anxiogenic effect, rat

Introduction

Nicotine is an alkaloid of tobacco which is abused by millions of people worldwide through cigarette smoking. Nicotine exerts rewarding/reinforcing effects via stimulation of the mesolimbic dopamine neurons and these effects have an important role in the induction of nicotine dependence by chronic administration of the drug (Balfour, 2009). Nicotine enhances midbrain dopamine transmission by the activation of NMDA receptors which are expressed on dopamine neurons of the ventral tegmental area (VTA) (Fu et al., 2000; Grillner and Svensson, 2000; Mansvelder and McGehee, 2000). Kenny et al. (2009) suggested that NMDA receptors of the VTA and the central amygdala (CeA) are involved in mediating the effects of nicotine on brain reward systems. Furthermore, most tobacco smokers report that nicotine alleviates negative moods such as anxiety and stress (Brody, 2006; Shiffman, 1993). In animal experiments, nicotine has been found to elicit both anxiolytic effects (Brioni et al., 1993, 1994; Szyndler et al., 2001) and anxiogenic effects (Biala and Budzynska, 2006; Biala and Kruk, 2009; Cheeta et al., 2001a). Ouagazzal et al. (1999) also reported that low doses of nicotine had no effect in experiments using the elevated plus-maze (EPM), but higher doses of the drug induced anxiogenic effects (Ouagazzal et al., 1999). Therefore, these discrepancies may be due to methodological issues such as animal species, the dose used and the time after drug testing or schedule of administration.

The anxiolytic or anxiogenic effects of nicotine may be due to alteration of release of brain neurotransmitters. For example, nicotine administration has been reported to increase glutamate (Lambe et al., 2003; Toth et al., 1992) which enhances anxiety-like behaviours (for review, see Bergink et al., 2004). In contrast, activation of nicotinic acetylcholine receptors induces release of gamma amino butyric acid (GABA) neurotransmission (Radcliffe et al., 1999) which may mediate nicotine-induced anxiolytic effects (Sullivan and Covey, 2002).

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Anxiety symptoms are among signs of psychiatric illness and are the most chronic of the mental disorders (Garakani et al., 2006). The incidences of anxiety worldwide account for billions of dollars of lost productivity annually. Thus, the neurobiological mechanisms of anxiety have received much attention. Several neurotransmitters such as GABA, glutamate, serotonin and noradrenaline have been suggested to be involved in anxiety (Bergink et al., 2004; Gordon and Hen, 2004; Moult, 2009). The neurotransmitter glutamate is considered to be an important element in anxiety (Cortese and Phan, 2005). Glutamate exerts its action by binding to specific membrane receptors. The glutamate receptor subtypes are divided into two major subclasses: ligand-gated ion channel (ionotropic) receptors, and G protein-coupled metabotropic receptors (Kew and Kemp, 2005). Ionotropic receptors – which are divided into the NMDA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and kainite subtypes – have been implicated in anxiety disorders (Chojnacka-Wojcik et al., 2001). Using a number of different classes of NMDA receptor antagonists in animal models of anxiety, such as the EPM, it was found that the blockade of these receptors can produce anxiolytic effects (for review, see Barkus et al., 2010). Furthermore, there is considerable evidence from preclinical animal studies and human drug trials that have suggested the therapeutic utility for glutamate receptor antagonists in treatment of anxiety (Cortese and Phan, 2005; Mathew et al., 2008).

Anxiety may be controlled by different brain structures, especially particular parts of the limbic system (Menard and Treit, 1999) including the amygdaloid complex (Davidson, 2002), which comprises several nuclei and has extensive connections with cortical and subcortical regions (Sah et al., 2003). Several lesion studies suggested the involvement of the amygdala in fear/anxiety conditioning. For example, in a study of the effects of NMDA-induced amygdala lesion, Strauss et al. (2003) reported the impairment of inhibitory avoidance, which indicates an anxiolytic effect. Among 13 nuclei of the amygdaloid complex (Pitkanen et al., 2000), it is well known that basolateral amygdala (BLA) and the CeA have critical roles in fear/anxiety-related behaviours (Rosen and Donley, 2006). A growing body of evidence suggests that the CeA is implicated in the modulation of anxiety-like behaviours in the EPM (Randall-Thompson et al., 2010; Rezayof et al., 2009; Uchiyama et al., 2008; Zarrindast et al., 2005, 2008a). Considering that the CeA is involved in anxiety-like behaviours and NMDA receptors of the CeA play a significant role in modulating emotional and behavioural states (Davis, 2006; Wang et al., 2007; Watanabe et al., 2002), the aim of the present study was to test whether the NMDA receptor mechanism of the CeA is also involved in nicotine-induced anxiety-like effect in rats. In the current study the EPM as used, which is a well known paradigm for evaluating anxiety-like behaviours in animals (Pellow et al., 1985).

Materials and methods

Subjects

Male Wistar rats bred in the department of pharmacology, Tehran University of Medical Sciences (Tehran, Iran), weighing 220–280 g at the time of surgery, were used. The animals were kept four per cage (42 × 26 × 15 cm height) under a 12/12 h light/dark cycle (lights on at 07:00 h) and controlled temperature (22 ± 2°C). They had free access to standard rat chow and tap water, and were allowed to adapt to the laboratory conditions for at least 1 week before surgery. Rats were handled for 5 min each day prior to the behavioural testing. Experimental groups consisted of eight animals and each animal was tested once. All experiments were performed between 10:00 h and 12:00 h. All procedures were performed in accordance with institutional guidelines for animal care and use.

Materials

Drugs (-)-nicotine hydrogen tartrate was purchased from Sigma-Aldrich. Doses are expressed as salts and were dissolved in saline (NaCl 0.9%) with pH adjusted to 7.4 with NaOH and injected intraperitoneally (i.p.) in a volume of 1 mL/kg. NMDA and D-(-)-2-amino-5-phosphonopentanoic acid (D-AP5) were purchased from Tocris Cookson Ltd, UK, and were dissolved in sterile saline. Bilateral microinjections of NMDA and D-AP5 into the central amygdala (Intra-CeA) were injected in a volume of 0.6 μL/rat (0.3 μL/side, bilaterally). The time intervals (Rezayof et al., 2010) and the doses used for systemic administration of nicotine (Zarrindast et al., 2010) and intra-CeA microinjections of NMDA receptor agents (Rezvanfard et al., 2009; Ardjmand et al., 2010) are based on our previous experiments and a pilot study.

Stereotaxic surgery and microinjections

The animals were anesthetized intraperitoneally with a ketamine/xylazine mixture (100 and 40 mg/kg, respectively) and placed in a stereotaxic frame (Stoelting Co., Illinois, USA). A midline scalp incision was made and the skin and underlying periosteum retracted. Then, two stainless-steel guide cannulas (22-gauge, Supa, Iran) were implanted bilaterally 1 mm above the center of the right and left CeA. The coordinates for the CeA were: incisor bar (−3.3 mm), 2.2 mm posterior to bregma; ± 4.1 mm lateral to the midline; and −8.1 mm from the top of the skull (Paxinos and Watson, 2007). The cannulas were anchored to the skull with a jeweler screw and dental cement, and then stainless steel stylets (27-gauge) were inserted into the guide cannulas to maintain patency prior to microinjections. The surgery was done with maximal care to minimize infection, and the animals were housed in a clean box after surgery. After surgery, the animals were allowed 7 days recovery before the behavioural testing.

For drug infusion, the animals were gently restrained by hand; the stylets were removed from the guide cannulas and replaced by 27-gauge injection needles (1 mm below the tip of the guide cannulas). Each injection unit was connected by polyethylene tubing to 2 μL Hamilton syringe. The left and right CeAs were injected with a 0.3 μL solution on each side (0.6 μL/rat) over a 60-s period. The injection needles were left in place for an additional 60 s to allow diffusion and then the stylets were reinserted into the guide cannulas. In cases of two injections, the second drug was injected 5 min after the first one.

Elevated plus-maze apparatus

The EPM apparatus was the same as described previously (Zarrindast et al., 2005, 2008b). The apparatus was a wooden cross-shaped maze consisting of four arms (two open arms and two closed arms), arranged in the shape of a plus sign. The open arms had no walls (50 × 10 cm), but to prevent the rats from falling, a rim of Plexiglas (0.5 cm high) surrounded the perimeter of the open arms. The other two arms were enclosed by walls 40 cm high (50 × 10 × 40 cm), except for the central part where the arms crossed. The whole apparatus was elevated 50 cm above the floor. Where the four arms intersected, there was a square platform of 10 × 10 cm. The room was illuminated by a 60-W bulb 1.5 m above the apparatus.

Behavioural testing

Seven days after the cannula implantations, the effects of intra-CeA injection of drugs were tested in the EPM. At least one hour before testing, the rats were placed in the experimental room. Eight rats were used in each experimental group. Animals were randomly allocated to treatment conditions and tested in counterbalanced order. The rats were individually placed in the center of the maze facing a closed arm and allowed free exploration for five minutes. The number of entries (all four paws into an arm) into open arms and closed arms, and the total time spent in the open arms and closed arms were recorded. The percentage of open arm time spent (%OAT) and open arm entries (%OAE) as the standard anxiety indices (Rodgers and Johnson, 1995) were calculated using the following formulae: (a) %OAT (the ratio of total times spent in the open arms to total times spent in the four arms × 100); (b) %OAE (the ratio of total entries into open arms to total entries in four arms × 100); and (c) total arms' entries were also recorded as a relative pure index of locomotor activity. The experimental sessions were recorded by a video camera interfaced with a monitor and a VCR in an adjacent room. Videotapes were scored by a highly trained observer, who was blind to treatment conditions.

Experiment 1: effects of nicotine on anxiety-like behaviours. In this experiment, five groups of animals received vehicle (1 mL/kg, i.p.) or different doses of nicotine (0.4, 0.5, 0.6 and 0.8 mg/kg, i.p.). The test session was performed 30 min after the drug injection, and %OAT, %OAE and locomotor activity were measured as described above.

Experiment 2: effects of intra-CeA microinjection of NMDA in the presence or absence of nicotine on anxiety-like behaviours. In this experiment, four groups of rats received vehicle (1 mL/kg, i.p.) 30 min before testing and vehicle (0.3 μL/side, bilaterally; 0.6 μL/rat) or different doses of NMDA (0.005, 0.05 and 0.1 μg/0.6 μl/rat) 5 min prior to intraperitoneal injection of vehicle. Four other groups of rats received nicotine (0.4 mg/kg, i.p.) 30 min before testing and saline (0.3 μL/side; 0.6 μL/rat) or three different doses of NMDA, 5 min before nicotine. During the behavioural testing, %OAT, %OAE and locomotor activity were measured as described above.

Experiment 3: effects of intra-CeA microinjection of D-AP5 in the presence or absence of nicotine on anxiety-like behaviours. In this experiment, five groups of rats received vehicle (1 mL/kg, i.p.) 30 min before testing and saline (0.3 μL/side, bilaterally; 0.6 μL/rat) or different doses of D-AP5 (0.01, 0.05, 0.1 and 0.5 μg/0.6 μL/rat) 5 min prior to intraperitoneal injection of vehicle. Four other groups of rats received nicotine (0.6 mg/kg, i.p.) 30 min before testing and vehicle (0.3 μL/side, bilaterally), or three different doses of D-AP5, 5 min before nicotine. During the behavioural testing, %OAT, %OAE and locomotor activity were measured as described above.

Experiment 4: effects of intra-CeA microinjection of D-AP5 on NMDA-induced anxiogenic-like effect. In this experiment, five groups of animals received intra-CeA microinjections of saline (0.6 μL/rat) or different doses of D-AP5 (0, 0.005, 0.0075 and 0.01 μg/rat) and after 5 min, they were injected with NMDA (0.1 μg/rat; intra-CeA). All animals received saline 5 min after NMDA administration. The test session was performed 30 min after final drug injection, and %OAT, %OAE and locomotor activity were measured as described above.

Histology

After completion of the experimental sessions, each rat was deeply anaesthetized and 0.6 μL/rat of a 1% methylene-blue solution was bilaterally microinjected into the CeA (0.3 μL/each side), as described in the drug section, then decapitated and its brain removed and placed in formaldehyde (10%). After several days, the brains were sliced and the sites of injections were verified according to Paxinos and Watson's atlas (Paxinos and Watson, 2007). Data from rats with incorrect placement were excluded from the analysis.

Statistical analysis

Since the distribution was normal, the data were analysed by analysis of variance (ANOVA). One-way ANOVA was used for comparison between the effects of different doses of nicotine. Two-way ANOVA was used for evaluation of interactions between drugs (nicotine and nicotine with either NMDA or D-AP5). Following a significant *F*-value, post-hoc analysis (Tukey-test) was performed to assess specific group comparisons. Differences with $p < 0.05$ between experimental groups at each point were considered statistically significant.

Results

Histology

Figure 1A shows the representative section taken from the rat brain atlas of Paxinos and Watson (2007). Figure 1B also

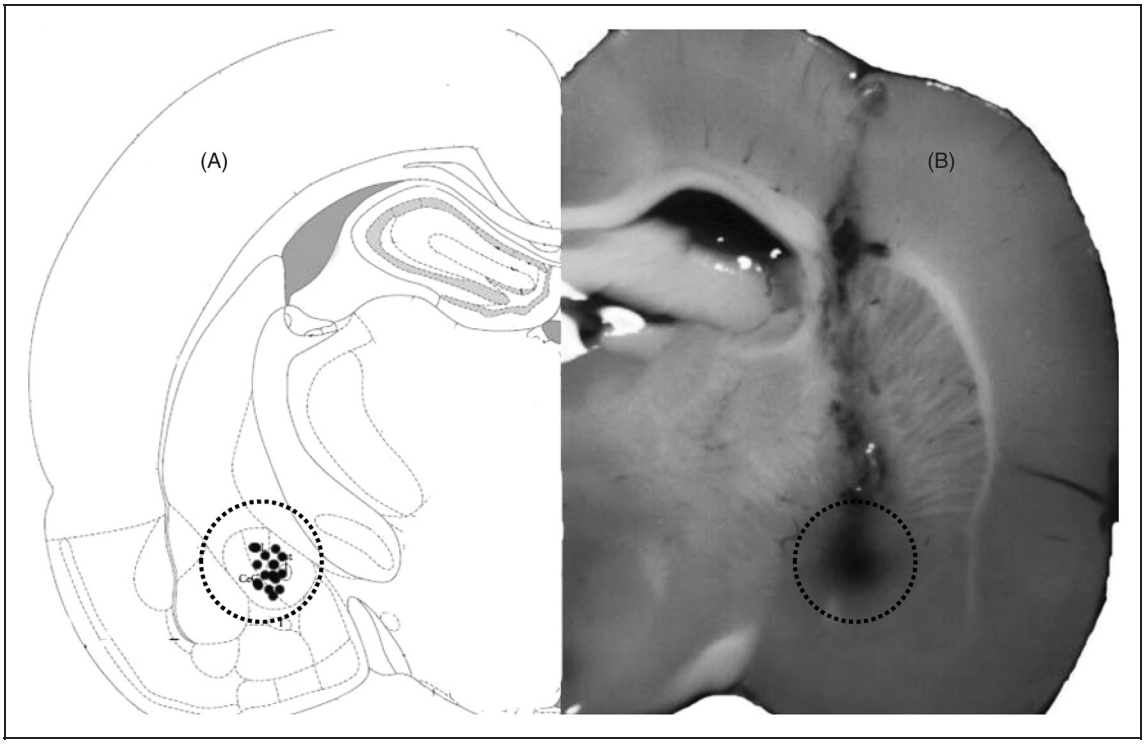

Figure 1. (A) Verified section of the central amygdala (CeA) was taken from the atlas of Paxinos and Watson, 2007. (Reproduced from Paxinos G and Watson C (2007) *The Rat Brain in Streretaxic Coordinates, 6th edition* with permission from Elsevier). (B) The location of the injection cannulae tips in the CeA regions for all rats were included in the data analyses.

shows the location of the injection cannula tip in the CeA. Shaded and dark areas represent the approximate points at which the cannula was positioned for each animal. Data from the animals with injection site located outside the CeA was not used in the analysis.

Effects of nicotine on anxiety-like behaviours

Figure 2 shows the effects of different doses of nicotine on anxiety-like behaviours. One-way ANOVA revealed that intraperitoneal administration of different doses of nicotine altered %OAT [F (4,35) = 9.3, p < 0.001] and %OAE [F (4,35) = 5.14, p < 0.01], but caused no change in locomotor activity [F (4,35) = 0.8, p > 0.05]. Post-hoc analysis showed that intraperitoneal administration of nicotine at doses of 0.6 and 0.8 mg/kg significantly decreased %OAT and %OAE, indicating anxiogenic-like effect.

Effects of intra-CeA microinjection of NMDA in the presence or absence of nicotine on anxiety-like behaviours

Figure 3 shows the effects of intra-CeA microinjection of NMDA alone or in combination with nicotine (0.4 mg/kg) on anxiety-related parameters in the EPM. Two-way ANOVA indicates a significant difference for %OAT [F (1,56) = 5.1, p < 0.05] and locomotor activity [F (1,56) = 4.9, p < 0.05], but not for %OAE [F (1,56) = 3.8, p > 0.05], between the response to NMDA (0.005, 0.05 and 0.1 μg/rat; intra-CeA) and that to NMDA plus nicotine (0.4 mg/kg; i.p.) with an interaction for %OAT [F (3,56) = 7.8, p < 0.001], but not for %OAE [F (3,56) = 0.4, p > 0.05] or locomotor activity [F (3,56) = 0.59, p > 0.05]. One-way ANOVA also revealed that intra-CeA administration of different doses of NMDA alone significantly decreased %OAT [F (3,28) = 17.9, p < 0.001] and %OAE [F (3,28) = 3.3, p < 0.05], but not locomotor activity [F (3, 28) = 0.6, p > 0.05]. Post-hoc analysis showed that intra-CeA administration of NMDA by itself at the doses of 0.05 and 0.1 μg/rat decreased %OAT, but not %OAE or locomotor activity. In addition, the lower dose of nicotine (0.4 mg/kg) alone did not induce a significant response. However, co-administration of the same doses of NMDA with the lower dose of nicotine (0.4 mg/kg) significantly altered %OAT [F (3, 28) = 5.03, p < 0.01] and %OAE [F (3,28) = 3.4, p < 0.05], but not locomotor activity [F (3,28) = 1.5, p > 0.05], indicating that nicotine reverses NMDA response. Further analysis showed that an intrinsically inactive dose of nicotine (0.4 mg/kg) significantly (p < 0.01) reversed the anxiogenic effect of intra-CeA administration of 0.1 μg/rat of NMDA for %OAT, but not for %OAE or locomotor activity.

Effects of intra-CeA microinjection of D-AP5 in the presence or absence of nicotine on anxiety-like behaviours

Figure 4 shows the effects of intra-CeA microinjection of D-AP5 alone or in combination with nicotine (0.6 mg/kg) on anxiety-related parameters in the EPM. Two-way ANOVA

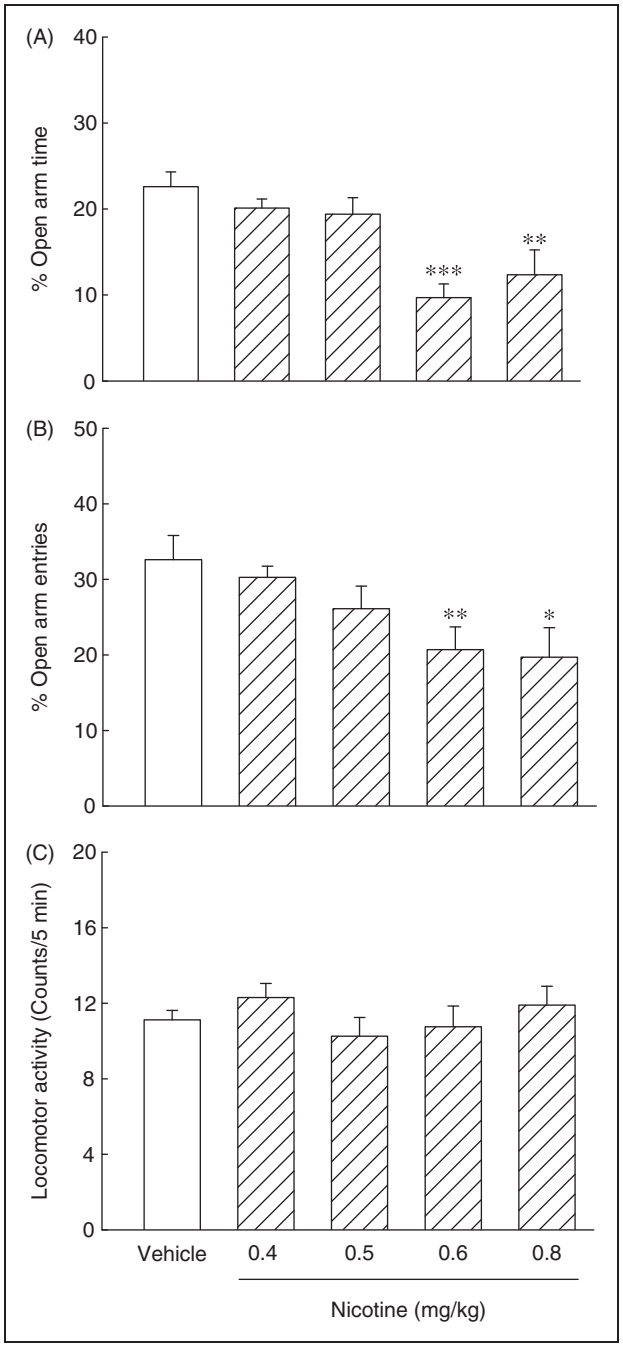

Figure 2. Effects of intraperitoneal administration of nicotine on rat behaviour in the elevated plus-maze (EPM). Rats were injected with nicotine (0, 0.4, 0.5, 0.6 and 0.8 mg/kg) 30 min before the EPM test. Each bar represents mean ± SEM of percentage open arm time spent (%OAT) (A), percentage open arm time entries (%OAE) (B) or locomotor activity (C). A total of 40 animals (8 rats in each group) was used in the experiment. Significant differences: $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$ compared to vehicle control group.

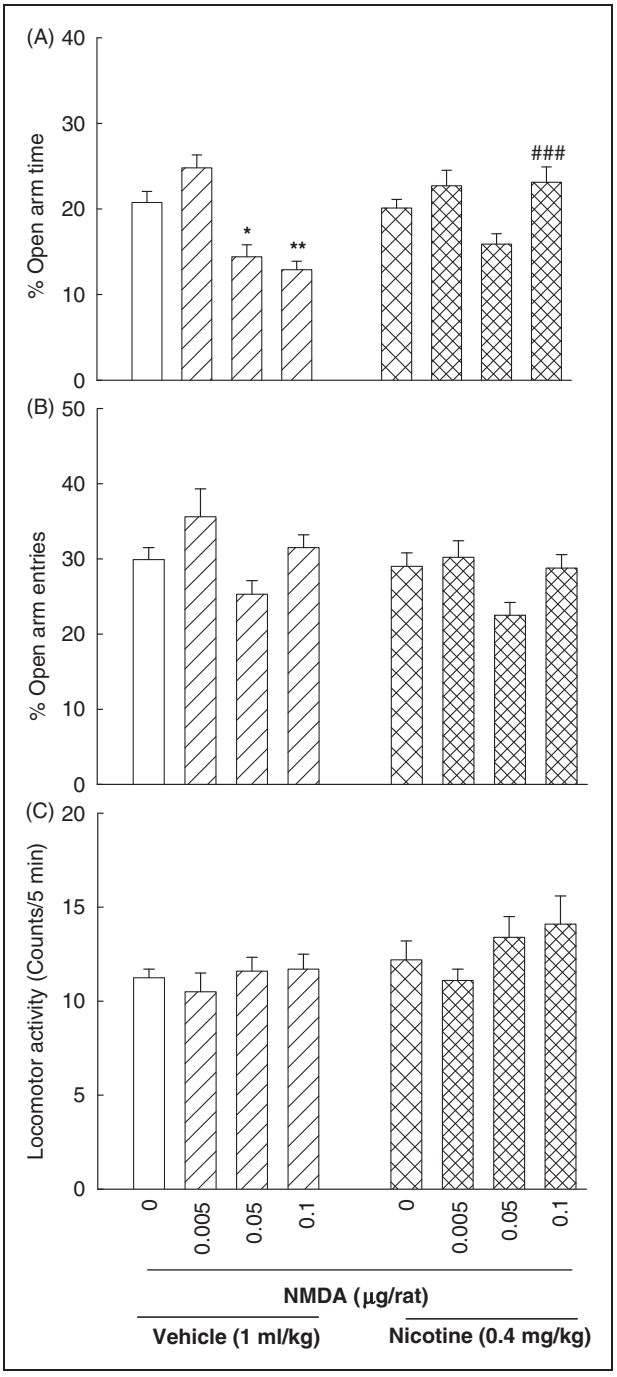

Figure 3. Effects of *N*-methyl-D-aspartate (NMDA) alone or with nicotine on rat behaviour in the EPM. The animals received intra-CeA microinjection of saline or different doses of NMDA (0.005, 0.05 and 0.1 μg/rat). After 5 min, they were injected with intraperitoneal injections of vehicle or nicotine, 30 min before testing. Each bar represents mean ± SEM of %OAT (A), %OAE (B) or locomotor activity (C). A total of 64 animals (8 rats in each group) was used in the experiment. Significant differences: $*p < 0.05$ and $**p < 0.01$, compared to saline control group; $###p < 0.001$ compared to the respective NMDA (0.01)/vehicle control group.

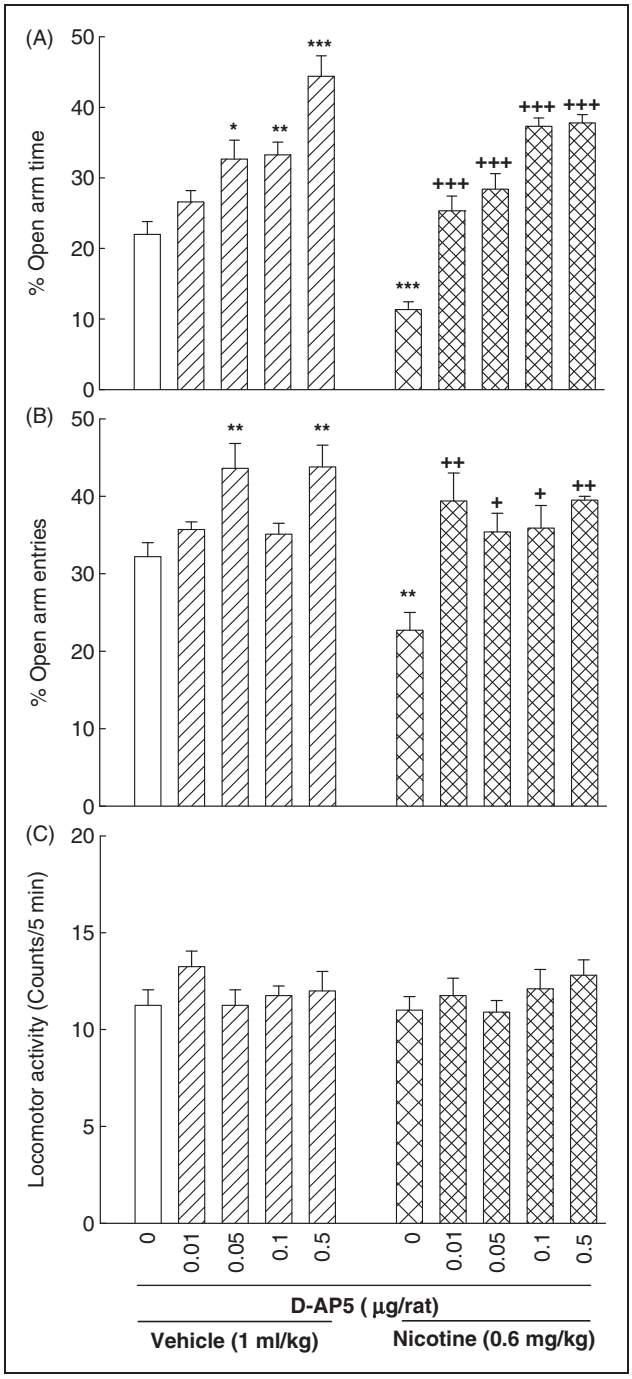

Figure 4. Effects of intra-CeA administration of D-AP5 alone or with nicotine on rat behaviour in the EPM. The animals received intra-CeA microinjection of saline or different doses of D-AP5. After 5 min, they were injected with intraperitoneal injections of vehicle or nicotine, 30 min before testing. Each bar represents mean ±SEM. of %OAT (A), %OAE (B) or locomotor activity (C). A total of 80 animals (8 rats in each group) was used in the experiment. Significant differences: $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$, compared to saline control group; $+p < 0.05$, $++p < 0.01$ and $+++p < 0.001$ compared to saline/nicotine group.

indicates a significant difference for %OAT [F (1,70) = 9.4, $p < 0.01$] and %OAE [F (1,70) = 5.4, $p < 0.05$], but not for locomotor activity [F (1,70) = 0.5, $p > 0.05$], between the response to D-AP5 (0.01, 0.05, 0.1 and 0.5 μg/rat; intra-CeA) and that to D-AP5 plus nicotine (0.6 mg/kg; i.p.) with an interaction for %OAT [F(4,70) = 4.0, $p < 0.01$] and %OAE [F (4,70) = 2.8, $p < 0.05$], but not for locomotor activity [F (4,70) = 0.4, $p > 0.05$]. One-way ANOVA also revealed that intra-CeA microinjection of different doses of D-AP5 (0.05, 0.1 and 0.5 μg/rat) alone increased %OAT [F (4, 35) = 14.34, $p < 0.001$], %OAE [F (4,35) = 5.7, $p < 0.01$], but not locomotor activity [F (4,35) = 1.1, $p > 0.05$]. Furthermore, one-way ANOVA also indicated that intra-CeA microinjection of D-AP5 reversed the anxiogenic-like effect of nicotine and increased %OAT [F (4,35) = 42.8, $p < 0.001$] and %OAE [F (4,35) = 7.3, $p < 0.001$], but not locomotor activity [F (4,35) = 0.52, $p > 0.05$].

Effects of intra-CeA microinjection of D-AP5 on NMDA-induced anxiogenic-like effect

Figure 5 shows the effect of intra-CeA microinjection of D-AP5 (0.005, 0.0075 and 0.01 μg/rat) on the response induced by NMDA (0.1 μg/rat) in the EPM. One-way ANOVA indicated that intra-CeA microinjection of D-AP5 reversed the effect of NMDA response in the EPM [for %OAT: F (4,35) = 19.6, $p < 0.001$, %OAE: F (4,35) = 7.6, $p < 0.001$, but not locomotor activity: F (4,35) = 1.1, $p > 0.05$]. Post-hoc analysis showed that the maximum effect was obtained by 0.01 μg/rat of D-AP5.

Discussion

In the present study, possible involvement of the NMDA subtype of glutamate receptors of the central amygdala (CeA) in the anxiogenic-like effect of nicotine has been investigated. The method of elevated plus-maze (EPM), which has been validated for anxiety-like behaviour (Pellow et al., 1985; Rodgers and Dalvi, 1997), was used here. The present data indicate that nicotine decreased percentage preference for open arm times (%OAT) and open arm entries (%OAE), parameters of anxiety behavior, indicating an anxiogenic-like effect for the drug in the EPM. Since nicotine did not show any significant effect on locomotion, the obtained results of the drug might be independent of locomotor activity or depressant effects of the drug. The obtained results are in line with our previous report showing that nicotine exerted an anxiogenic response (Zarrindast et al., 2000; Zarrindast et al., 2010). However, there is no general consensus on nicotine response in animal models of anxiety, but there is evidence that nicotine can elicit anxiolytic-like behaviour (Brioni et al., 1993). Another investigation suggests that the alkaloid is ineffective in low doses and can induce anxiogenic-like behaviours in high doses (Ouagazzal et al., 1999). On the other hand, it has been reported that nicotine exerts both anxiogenic-like and anxiolytic-like activity based on the dose range used (File et al., 1998), gender difference (Cheeta et al., 2001b) and method of testing (Cheeta et al., 2001a).

It is well established that systemic nicotine exposure alters the levels of some neurotransmitters in the brain. For example,

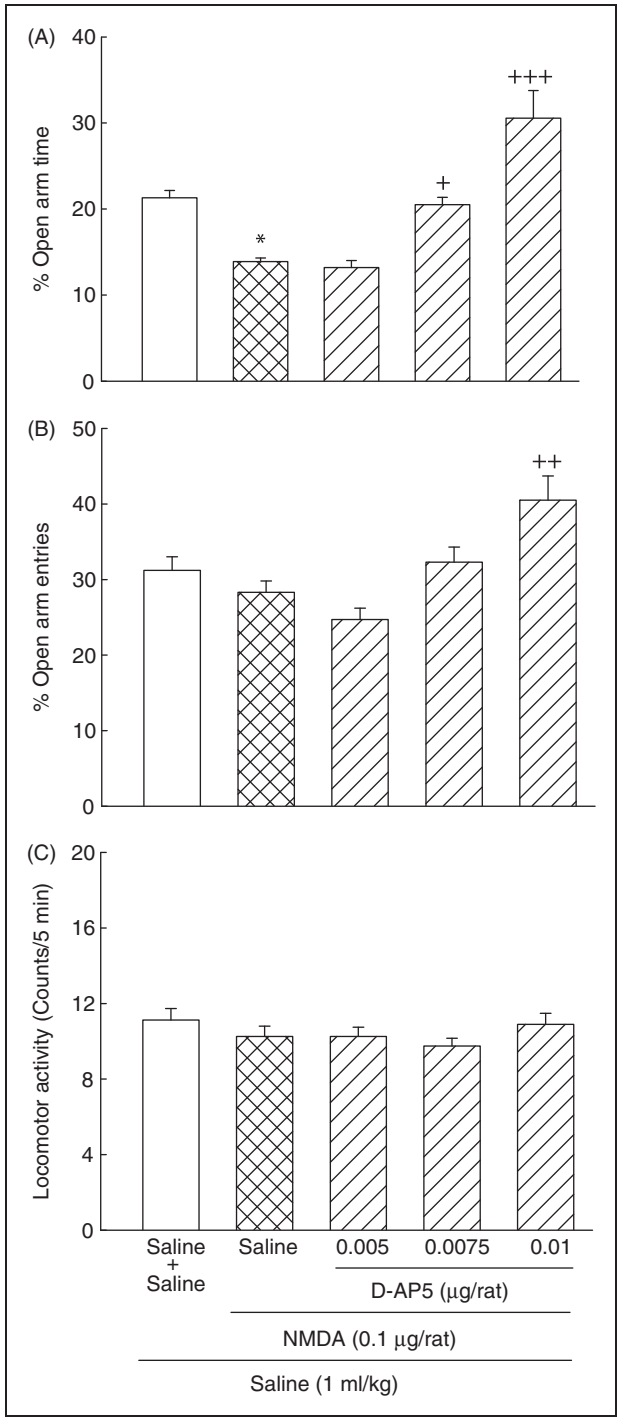

Figure 5. Effect of D-AP5 on the response induced by NMDA in the EPM. The animals received bilateral intra-CeA microinjection of D-AP5 and after 5 min they were injected with NMDA. All animals received saline 5 min after NMDA administration. The control groups also received two or three saline injections. Each bar represents mean ± SEM of %OAT (A), %OAE (B) or locomotor activity (C). A total of 40 animals (8 rats in each group) was used in the experiment. Significant differences: $*p < 0.05$, compared to saline/saline/saline control group; $+p < 0.05$, $++P < 0.01$ and $+++p < 0.001$ compared to saline/NMDA/saline control group.

it has been shown that systemic administration of nicotine enhances the ratio of dopamine neuronal currents (Gao et al., 2010) and GABAergic synaptic transmission (Nakamura and Jang, 2010). Furthermore, nicotine can stimulate the release of glutamate (Fallon et al., 2007; Lambe et al., 2003) which is an important factor in defensive behaviours (Bergink et al., 2004; Carobrez et al., 2001; Javitt, 2004), and it has also been suggested that nicotine may increase glutamate-mediated transmission in the amygdala (Barazangi and Role, 2001). Considering that NMDA receptors of the amygdala are involved in anxiety processes (Strauss et al., 2003) and the CeA has a key role in mediating nicotine effects (Yamada and Bruijnzeel, 2010), the present study aimed to explore the role of NMDA receptors of the CeA in nicotine-induced anxiogenic-like behaviours. Our data showed that microinjections of NMDA into the CeA decreased %OAT and %OAE, but not locomotor activity, indicating anxiogenic-like effect. A large body of evidence suggests the involvement of NMDA receptors in the neurobiology of emotion including fear, anxiety and depression (Barkus et al., 2010). There is a report indicating that interference with NMDA receptors of the hippocampus modified the anxiogenic effect of stress in the EPM (Padovan et al., 2000). The present results are in agreement with a report showing the mice that lack the NR1 subunit of the NMDA receptors in the dendate gyrus (NR1 knockout mice) were less anxious (Niewoehner et al., 2007). Moreover, our data show that co-administration of the same doses of NMDA with the lower dose of nicotine, which did not induce a significant response by itself, significantly changed %OAT and %OAE with an interaction. One may suggest that the CeA NMDA receptors are involved in mediating nicotine's effect. However, post-hoc analysis could not show a distinct difference between the groups which received different doses of NMDA. It should be noted that a functional interaction between nicotine and glutamate in cognitive functions such as learning and memory has been suggested both in rodents (Ciamei et al., 2001; Rezvani and Levin, 2003) and humans (Jackson et al., 2009). Therefore, it seems that glutamate may have a critical role in neurobiological mechanisms underlying nicotine functions. Due to release of different neurotransmitters by nicotine which may influence anxiety differently and the opposite effects of the drug on anxiety by itself, it is impossible to discuss the inhibition of NMDA response by nicotine at the moment. Therefore, this issue remains to be clarified later.

Moreover, the effects of NMDA receptor antagonist on emotional processing in rodents have been extensively described in the literature. The NMDA receptor antagonists have been shown to have anti-anxiety properties (Barkus et al., 2010). The present study showed that intra-CeA microinjection of the NMDA receptor antagonist, D-AP5, increased %OAT and %OAE by itself. Moreover, our results indicated that intra-CeA microinjection of the ineffective doses of D-AP5 reversed the NMDA-response in the EPM. The increases in these parameters of anxiety tests reveal an anxiolytic-like effect, which has been shown by other reports (Fallon et al., 2007). Our obtained results are in agreement with those indicating that non-competitive NMDA receptor antagonists, phencyclidine and ketamine, elicited anxiolytic responses in mice (Porter et al., 1989) and rats (Wiley et al., 1995), respectively. In the present study, when D-AP5 was microinjected into the CeA prior to nicotine, it altered nicotine's anxiogenic

response. However, on the basis of the statistical analysis, it has been suggested that intra-CeA microinjection D-AP5 reversed the anxiogenic-like effect of nicotine; one may also suggest that the anxiogenic effect of nicotine and the anxiolytic effect of D-AP5 could be entirely independent phenomena. It has been reported that the administration of the NMDA receptor antagonist dizocilpine could attenuate the positive effects of nicotine on memory consolidation (Ciamei et al., 2001). There is also growing evidence from behavioural studies to show that systemic administration of NMDA receptor antagonists can decrease nicotine self-administration and the nicotine discriminative stimulus (Blokhina et al., 2005; Glick et al., 2001; Wright et al., 2006; Zakharova et al., 2005). Furthermore, it has been reported that microinjection of the competitive NMDA receptor antagonist LY235959 into the CeA or the VTA decreased nicotine self-administration behaviour in a dose-dependent manner (Kenny et al., 2009). Therefore, our final conclusion may be that glutamate-mediated transmission in the CeA is necessary for nicotine functions.

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