Modulation of ventral tegmental area dopamine receptors inhibit nicotine-induced anxiogenic-like behavior in the central amygdala

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A R T I C L E  A B S T R A C T

Article history:
Received 13 May 2012
Received in revised form 5 September 2012
Accepted 14 September 2012
Available online 21 November 2012

Keywords:
Anxiety
Central amygdala
Elevated plus maze
Nicotine
Ventral tegmental area

Nicotine, the major addictive substance in tobacco, increases the activity of the central amygdala (CeA). Amygdala is directly implicated in anxiety modulation and sends projections to the vicinity of the midbrain dopamine neurons, including the ventral tegmental area (VTA) which is a key area that controls nicotine dependence processes. In this study, the role of dopamine D1 and D2/3 receptors of the VTA on anxiogenic-like behavior induced with intra-CeA injection of nicotine has been investigated. Male Wistar rats with cannula aimed to the left CeA and the left VTA were submitted to the elevated plus-maze (EPM). The nicotine injection (1 μg/rat) into the CeA decreased the percentage of open arm time and open arm entries, but not locomotor activity, indicating an anxiogenic-like response. Intra-VTA injection of a dopamine D1 receptor antagonist, SCH23390 (0.25 μg/rat), and a dopamine D2/3 receptor antagonist, sulpiride (0.7 μg/rat), inhibited the anxiogenic-like response caused by intra-CeA injection of nicotine. These results suggest that the relationship between the VTA and the CeA may be involved in nicotine-induced anxiogenic-like behavior via dopamine D1 and D2/3 receptors. An understanding of these cellular processes will be crucial for the development of new intervention to combat nicotine effect.

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1. Introduction

Nicotine, the major addictive substance in tobacco increases the activity of reward related brain areas, such as the central amygdala (CeA) and the nucleus accumbens (Brunzell et al., 2003; Pagliusi et al., 1996; Pich et al., 1997). It is believed that amygdala directly modulates anxiety (Kalin et al., 2004; Lesscher et al., 2008) and provides the main input to the dopamine neurons (Cassell et al., 1986; Hopkins, 1975; Price and Amaral, 1981). The CeA also sends projections to the vicinity of the midbrain dopamine neurons (Fudge and Haber, 2000; Wallace et al., 1992), including the ventral tegmental area (VTA), which is a key region that regulates nicotine dependence processes (Corrigall et al., 1994; David et al., 2006; Ikemoto et al., 2006). Furthermore, nicotine increases the firing rate of VTA dopamine neurons (Grenhoff et al., 1986; Li et al., 2011; Schistrom et al., 2003) and modulates anxiety (Renowitz, 2008). The expression of nicotinic acetylcholine receptor (nAChR) alpha2 subunit in rat amygdala has been reported (Ishii et al., 2005). Central nAChRs are particularly presynaptic and exert a modulatory influence (Role and Berg, 1996) on neurotransmitter release (Dajas-Bailador and Wonnacott, 2004). For instance, the activation of presynaptic nAChRs modulates glutamatergic and GABAergic synaptic transmission in the amygdala (Barazangi and Role, 2001).

In most studies, nicotine is administered systemically or peripherally (for review see Matta et al., 2007), whereas central administration studies in animals help identify the involvement of specific brain area in the anxiogenic and anxiolytic actions of nicotine. While, there is a report of interaction between the CeA and the VTA in the acquisition of conditioned cue-directed behavior (Lee et al., 2011), little is known about the interaction between the CeA and the VTA on anxiety-related behavior in the EPM test.

Dopamine is a key neurotransmitter involved in reward (Koob, 1992) and dopamine receptors consist of two families, D1-like (D1 and D5) and D2-like (D2, D3 and D4) (Sibley et al., 1993). Dopamine D1 receptors are expressed in moderate to low density in the VTA (Ariano et al., 1997) and the local perfusion of D1 receptor agonists in the VTA increases the local release of both glutamate and GABA (Kalivas and Duffy, 1995), which can control the activity of dopamine neurons (Adell and Artigas, 2004). Furthermore, the dopamine D2...
receptors are highly expressed in the VTA of rodents (Mansour et al., 1990; Wamsley et al., 1989). Activation of D2 autoreceptors leads to increased potassium conductance that hyperpolarizes the plasma membrane of dopaminergic neurons (Adell and Artigas, 2004). It has been shown that dopamine dendritic release regulates the firing rate of dopaminergic neurons in the VTA through D2 receptors located in the glutamatergic nerve ending (Koga and Momiyama, 2000).

In the present study, the possible involvement of dopamine D1 and D2 receptors of the VTA on anxiogenic-like behavior induced with intra-CeA injection of nicotine has been investigated. The SCH23390, as a dopamine D1 antagonist (Hyttel, 1983; Iorio et al., 1983), and sulpiride as a dopamine D2 antagonist (Rüther et al., 1999) have been used. The anxiety parameters have been assessed by the elevated plus-maze (EPM) task. According to the definition of Pellow and File in 1986, an anxiolytic drug is the one that increases the number of entries into and the time spent in the open arms and the opposite state is true by an anxiogenic drug.

2. Experimental procedures

2.1. Drugs

Nicotine hydrogen tartrate was purchased from Sigma (Poole, Dorset, UK), SCH23390 from Tocris, UK and sulpiride from Sigma Chemical Company (St. Louis, CA, USA). Immediately before the experiment, SCH23390 and nicotine were dissolved in sterile normal saline. Sulpiride was dissolved in one drop of glacial acetic acid and made up to a volume of 5 ml with sterile 0.9% saline and then diluted to the required concentration. The pH of the solutions was adjusted to 7.2 with NaOH (0.1 N solution). The volume of the drug injection was 0.2 μl/rat. Drug total doses were expressed as μg/rat. Saline was injected to control animals instead of drug solution.

2.2. Animals

The study was performed on male Wistar rats weighing between 220 and 250 g at the time of the surgery which were housed in groups of 2–3 per cage on a 12/12 h light/dark cycle. All rats received standard laboratory rat chow and water ad libitum. A total of eight animals were used in each group (n=8) and each animal was used only once. All procedures were conducted in adherence with the institutional guidelines for animal care and use. The Research and Ethics Committee of the School of Medicine, Tehran University of Medical Sciences approved the experimental protocols.

2.3. Surgery and microinjection

The animals were anesthetized with 50 mg/kg ketamine hydrochloride plus 4 mg/kg xylazine intraperitoneally and placed on a stereotaxic frame, and the skull was exposed. The stainless-steel guide cannula was directed toward the left VTA and the left CeA. It was fixed with polycryl cement anchored to the skull with stainless-steel screws. Coordinates for cannulae implantation (Paxinos and Watson, 2007) in the VTA were: anterocaudal: −5.8 mm; lateral: ±0.7 mm (both with respect to bregma); and vertical: 8 mm (from dura), and coordinates for the CeA were: anterocaudal: −2.3 mm; lateral: ±4.1 mm (both with respect to bregma); and vertical: 8.1 mm (from dura). The animals were allowed 7 days to recover before the test. For drug injection, the animals were held gently, and the injection cannula extending 1.0 mm beyond the ventral tip of the implanted guide cannula was gently inserted into each cannula and connected with polyethylene tubing to a 1-μl Hamilton syringe. The drug solution was injected over 1 min. Each rat received 0.2 μl injection solution into the CeA and the VTA on the left side. After the injection, the injection cannulae were left in place for an additional 1 min in order to minimize leakage up the cannula track.

2.4. Behavioral testing (elevated plus maze)

Measurements were conducted according to the method of Pellow and File, 1986. The wooden tool consisted of two open arms (50 cm × 10 cm), and two closed arms of the same size but with 40 cm high end and side walls. The arms were linked by a central 10 × 10 cm area. After 1 hour adaptation to the testing room, the rats were located in the center of the plus maze with their head facing an open arm and left undisturbed for 5 min. Before the next rat was tested, the maze was cleaned with a 10% chlorine bleach solution and dried with a cloth.

The experimental sessions were recorded on tape and analyzed later. A rat was considered to be on the central platform when at least two paws were on it and on an arm whenever all four paws were on it. Percent time spent in open arms [%OAT: (time in open arm/time in “open+closed” arm) × 100] and percent of open arm entries [%OAE: (number of open arm entries/number of “open+closed” arm entries) × 100] were considered as an anxiety index. The spontaneous locomotor activity was calculated with the number of total arm entries.

2.5. Experimental procedure

2.5.1. Experiment 1: Intra-CeA injection of nicotine on anxiety-related behavior in the EPM test

In the first experiment, after 5 minute saline injection into the VTA, the different doses of nicotine (0.5, 1 and 1.5 μg/rat) were injected into the CeA. The percent time spent in open arms [%OAT], the percent of open arm entries [%OAE] and the locomotor activity were measured 5 min after the injections.

2.5.2. Experiment 2: Intra-VTA injection of sulpiride on anxiety-related behavior in the EPM test in the presence or absence of nicotine

The rats received intra-VTA injection of different doses of sulpiride (0.2, 0.3, 0.5, 0.7 and 1 μg/rat) in combination with intra-CeA injection of an effective dose of nicotine (1 μg/rat) or saline.

2.5.3. Experiment 3: Intra-VTA injection of SCH23390 on anxiety-related behavior in the EPM test in the presence or absence of nicotine

The rats received intra-VTA injection of different doses of SCH23390 (0.125, 0.25, 0.5 μg/rat) in combination with intra-CeA injection of saline. In part 2, the rats received intra-VTA injection of effective (0.125, 0.5 μg/rat) and sub-effective (0.25 μg/rat) doses of SCH23390 in combination with intra-CeA injection of an effective dose of nicotine (1 μg/rat).

Experimental groups:

<table>
<thead>
<tr>
<th>Drug</th>
<th>Group</th>
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<tbody>
<tr>
<td>Intra – VTA saline 3.10^(-5)</td>
<td>Intra – CeA nicotine (0.5, 1, 5 μg/rat)</td>
</tr>
<tr>
<td>Intra – VTA nicotine (0.5, 1, 5 μg/rat)</td>
<td>Intra – CeA saline</td>
</tr>
<tr>
<td>Intra – VTA saline (0.5, 1, 5 μg/rat)</td>
<td>Intra – CeA sulpiride</td>
</tr>
<tr>
<td>Intra – VTA sulpiride (0.2, 0.3, 0.5, 0.7 μg/rat)</td>
<td>Intra – CeA saline</td>
</tr>
<tr>
<td>Intra – VTA saline (0.5, 1, 5 μg/rat)</td>
<td>Intra – CeA saline</td>
</tr>
<tr>
<td>Intra – VTA salpinide (0.2, 0.3, 0.5 μg/rat)</td>
<td>Intra – CeA saline</td>
</tr>
</tbody>
</table>

2.6. Histology

At the end of the experimental period the drug injection site was verified by methylene blue (1%) injection in the same volume as drug injections. The animals were sacrificed with anesthetic overdose, their brains were removed and injection sites were verified histologically according to Paxinos and Watson (2007) atlas. Cannulae were implanted for a total of 204 animals, but only the data from 192 animals with correct cannulae implants were included in the statistical analyses.
2.7. Statistical analysis

Analysis of the data was performed using one-way analysis of variance (ANOVA). Following a significant F-value, Tukey post-hoc analysis test was performed to assess specific group comparisons. The null hypothesis was rejected at the 0.05 level of significance. Data were expressed as mean ± SEM.

3. Results

Fig. 1 shows the approximate point of the drug injections in the CeA and the VTA. The histological results were plotted on the representative section taken from the rat brain atlas of Paxinos and Watson (2007). Data from the animals with the injection sites located outside the CeA and the VTA were not used in the analysis.

3.1. Effects of three doses of nicotine on rat behavior in the EPM

As shown in Fig. 2, the intra-CeA injection of nicotine (1 μg/rat) exerted a significant reduction in %OAT \( F(3, 28)=13.1, P<0.001 \) and %OAE \( F(3, 28)=5.26, P<0.05 \) compared to saline + saline group. No significant change in the locomotor activity \( F(3, 28)=0.4, P>0.05 \) was observed. The data showed that the maximum effect was obtained with 1 μg/rat of nicotine, while the injection of 0.5 and 1.5 μg/rat of nicotine had no significant effect on the anxiety-related parameters in the EPM.

3.2. Effects of sulpiride on rat behavior in the EPM

As shown in Fig. 3, intra-VTA injection of sulpiride (0.2, 0.3, 0.5, 0.7 and 1 μg/rat) had no significant effect on %OAT \( F(5, 42)=0.91, P>0.05 \), %OAE \( F(5, 42)=2.79, P>0.05 \) and locomotor activity \( F(5, 42)=0.79, P>0.05 \) compared to saline + saline group.

Intra-VTA injection of sulpiride (0.7 μg/rat) with intra-CeA injection of an effective dose of nicotine (1 μg/rat) significantly increased %OAT \( F(4, 35)=8.41, P<0.001 \) and %OAE \( F(4, 35)=3.87, P<0.05 \) compared to saline + nicotine group. As shown in Fig. 4, locomotor activity \( F(4, 35)=0.506, P>0.05 \) showed no significant changes compared to saline + nicotine group (Fig. 4).

3.3. Effects of SCH23390 on rat behavior in the EPM

In Fig. 5, the effects of intra-VTA injection of different doses of SCH23390 (0.125, 0.25, 0.5 μg/rat) were shown. The dose of 0.5 μg/rat significantly increased %OAT \( F(3, 28)=4.8, P<0.05 \) and %OAE \( F(3, 28)=3.2, P<0.05 \) compared to saline + saline group. The treatment had no effect on the locomotor activity \( F(3, 28)=0.27, P>0.05 \). It should be considered that the dose of 0.125 and 0.25 μg/rat had no significant effect on the %OAT and %OAE compared to saline + saline group.

Intra-VTA injection of sub-effective dose of SCH23390 (0.25 μg/rat) with intra-CeA injection of an effective dose of nicotine (1 μg/rat) significantly increased %OAT \( F(4, 35)=21.34, P<0.01 \) and %OAE \( F(4, 35)=11.82, P<0.01 \) compared to saline + nicotine group. The response of the antagonist not only inhibited anxiogenic-like effect of nicotine, but also produced an anxiolytic-like effect. No significant changes were shown in locomotor activity \( F(4, 35)=0.67, P>0.05 \) compared to saline + nicotine group (Fig. 6).
4. Discussion

The present findings indicate that nicotine injection into the central amygdala (CeA) decreased % open arm time spent (%OAT) and % open arm entries (OAE %) in the elevated plus maze (EPM) test, suggesting an anxiogenic-like effect. Although, this is consistent with previous studies showing an anxiogenic-like effect following the systemic administration of nicotine (Foulds et al., 1997; Plaza-Zabala et al., 2010; Zarrindast et al., 2010), there is less investigation regarding the effects of central administration of nicotine on anxiety-related behavior (Zarrindast et al., 2008). In the present experiment, nicotine was infused into the CeA and a U-shaped dose–response curve was observed. The maximum effect was obtained with 1 μg/rat of nicotine, which was considered as a nicotine effective dose. When peripherally administered, nicotine can exhibit an inverted U-shaped dose–response curve (for review see Picciotto, 2003). It seems that low and higher doses of nicotine produce anxiolytic- and anxiogenic-like effects respectively (for review see Matta et al., 2007). The effects of nicotine are complex and there are certain discrepancies between studies, which may be attributable, in part, to the different doses, injection sites, or routes of nicotine administration.

Moreover, amygdala has a direct role in anxiety modulation (Kalin et al., 2004; Lesscher et al., 2008; Roozendaal et al., 2009) and there is evidence indicating anxiogenic and anxiolytic effects following the intra-amygdala infusion of D1 agonists and antagonists respectively (de la Mora et al., 2010). Nicotine has long been known to modulate synaptic transmission and plasticity in the amygdala (Fu et al., 1998; Mansvelder et al., 2009) and acute nicotine has been shown to increase c-fos mRNA expression, a marker for neuronal activation (Herrera and Robertson, 1996), in many brain regions, including the CeA (Salminen et al., 1999).

Considering the release of dopamine by nicotine (Di Chiara, 2000; Fu et al., 2000; Markou, 2008; Picciotto, 1998), it may be possible that intra-CeA injection of nicotine induces anxiogenic-like effect directly or indirectly through the dopamine receptors.
Several studies reported that the amygdala provides the main input to dopamine neurons (Hopkins, 1975; Price and Amaral, 1981). Dopamine secretion within the nucleus accumbens through the VTA stimulation is essential for the nicotine reinforcing effects (Corrigall et al., 1992). It has been reported that electrical stimulation of the VTA caused neural discharge in the CeA and a fundamental link between the VTA activation and neural excitability in the CeA, has been suggested (Gelowitz and Kokkinidis, 1999). However, experimental support for the CeA and the VTA interaction in nicotine-induced anxiety has been scarce.

In the present study, intra-CeA injection of nicotine showed an anxiogenic-like response which was inhibited by the intra-VTA injection of sulpiride. It has been shown that dopamine can be released from the cell body and dendrites in addition to axon terminals (Björklund and Lindvall, 1975). Dopamine containing neurons in VTA are about 60% (Margolis et al., 2006). Moreover, the extracellular pool of dopamine can activate local inhibitory D$_2$ autoreceptors (White and Wang, 1984) which are highly expressed in the VTA of rodents (Mansour et al., 1990; Wamsley et al., 1989). Activation of the D$_2$ autoreceptors leads to increased potassium conductance that hyperpolarizes the plasma membrane of dopaminergic neurons (Adell and Artigas, 2004). It has also been shown that, 89% of dopaminergic neurons and 40% of non-dopamine neurons that project to the NAc are inhibited by D$_2$ receptor activation (Yim and Mogenson, 1980). However, Margolis et al. (2008) showed that amygdala-projecting VTA dopamine neurons are not inhibited by D$_2$ receptor agonists and it is unlikely that intra-VTA injection of sulpiride in the present study could enhance dopamine impulse flow of meso-amygdala dopamine cells.

Under control conditions, the VTA dopaminergic neurons receive balanced inhibitory and excitatory inputs (Yang et al., 2009). Nicotine...
activates CeA (FU et al., 1998; Mansvelder et al., 2009; Salminen et al., 1999), and activation of presynaptic nAChRs modulates glutamatergic and GABAergic synaptic transmission (Barazangi and Role, 2001). In addition, the CeA projects to the vicinity of dopamine cell bodies in VTA (Fudge and Haber, 2000; Wallace et al., 1992) which control the mesocorticolimbic dopamine system (Phillips et al., 2003). It may be predicted that intra-CeA nicotine injection in the present study leads to a change of output from the CeA to VTA. However, whether the intra-CeA nicotine injection leads to a reduction in VTA dopamine impulse flow is not clear and this issue should be clarified by further experiments. The somatodendritic dopamine release in the mesocorticolimbic system is mainly dependent on the nerve impulse activity in the VTA (Adell and Artigas, 2004; Chen and Rice, 2002; Rice et al., 1997) and regulates the firing rate of dopaminergic neurons in the VTA through D2 receptors located in the glutamatergic nerve ending (Koga and Momiyama, 2000). In the presence of sulpiride, the roles of D2 receptors are blocked. We suggest that sulpiride ability to inhibit aminergic activity of nicotine may be related to inactivation of local inhibitory D2 autoreceptors. Furthermore, in the present study, intra-VTA injection of 0.25 μg/rat of SCH23390 which had no effect by its own increased %OAT and %OAE and hence blocked the anxiogenic effect of intra-CeA nicotine injection. The response of the antagonist not only inhibited the anxiogenic effect of nicotine, but also produced an anxiolytic effect. Local perfusion of D1 receptor agonists in the VTA increases the local release of both glutamate and GABA (Kalivas and Duffy, 1995), which can control the activity of dopamine neurons (Adell and Artigas, 2004). The activation of D1 receptors located on the VTA dopaminergic neurons or non-dopaminergic nerve terminals increases D1 receptor-mediated inhibition via inhibitory neurons (Momiyama et al., 1993). Therefore, the effects of SCH23390 and sulpiride in the present study were in the same direction.

Moreover, serotonergic neurons modulate the function of dopamine neurons through the 5-HT receptor subtype, which is also expressed in the VTA. It has been shown that 5-HT2 receptor agonists block the stimulatory action of nicotine on midbrain dopamine function and thus reduce its reinforcing effects (Fletcher et al., 2008; Lanteri et al., 2008). Furthermore, SCH23390 is also a potent agonist at 5-HT2c receptors (Millan et al., 2001), therefore, its counteract nicotine effect in the present study, may be attributable, in part, to its effect on VTA 5-HT2c receptors.

Although, the present results suggest the involvement of the dopamine transmission, through D1 and D2/3 receptors of the VTA in the anxiogenic-like effect of nicotine in the EPM task, we should take into account the interaction between the VTA and the nucleus accumbens to the development of nicotine dependence. The nucleus accumbens receives dopaminergic neurons from the midbrain VTA which implicated in motivation and reinforcement. There is also a suggestion that a significant number of nucleus accumbens medium spiny neurons project back to the observed effect in the present study may be modulated by the communication of the VTA and the nucleus accumbens.

The elevated plus-maze (EPM) has become one of the most popular rodent models of anxiety (Carobrez and Bertoglio, 2005; Pellow et al., 1985) to screen anxiolytic effects of drugs (Lister, 1992; Back and Surve, 2001; Xie et al., 2011). Therefore, the observed effect in the present study may be modulated by the communication of the VTA and the nucleus accumbens.

5. Conclusion

The results of the present study demonstrate that the anxiogenic-like effect of intra-CeA injection of nicotine may be mediated through activation of dopamine D1 and D2/3 receptors in the VTA. Such a relation should be considered in the modulation of anxiety in nicotine effects.

Acknowledgments

The authors thanks the IRAN National Science Foundation (INSF) for providing the financial support for the project.

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