Role of α7 nicotinic receptors in nicotine dependence and implications for psychiatric illness

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Accepted 10 January 2000

Abstract

It has previously been shown that the reinforcing and dependence-producing properties of nicotine depend to a great extent on activation of nicotinic receptors within the ventral tegmental area (VTA), i.e. the site of origin of the mesolimbocortical dopaminergic projection. Based on the data reviewed in the present study, it is suggested that nicotine by stimulating presynaptic α7 nicotinic receptors within the VTA, that are probably localized on glutamatergic afferents from the medial prefrontal cortex, produces sequentially an increase in glutamate concentrations, stimulation of NMDA receptors found on dopamine (DA)-containing neurons in the VTA, enhanced firing activity of VTA-DA neurons, augmented DA release in the nerve terminal regions, and enhanced c-fos expression in the dopaminergic projection areas through activation of D1-DA receptors. In addition, it appears that α7 nicotinic receptors within the VTA are directly involved in nicotine-related reward and withdrawal responses. These data may be instrumental in understanding how nicotine interacts with the mesolimbocortical dopaminergic system, which is perhaps the most important component of the neural mechanisms underlying nicotine dependence. These results may also contribute to unraveling the cellular basis of nicotine’s association with neuropsychiatric disorders, thereby offering the prospect of new therapeutic advances for their treatment. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Dopamine; Mesolimbocortical; Methyllycaconitine; Medial prefrontal cortex; Nucleus accumbens; Ventral tegmental area

1. Nicotine’s dependence liability and mesolimbocortical dopaminergic function

Nicotine-dependent, habitual smokers fulfil most, if not all, of the diagnostic criteria for substance dependence as defined by the DSM-IV, i.e. the diagnostic classification manual published by the American Psychiatric Association. There is ample evidence that the reinforcing, behavior-stimulating and dependence-producing properties of nicotine depend to a great extent on activation of the mesolimbocortical dopaminergic system, that consists of dopamine (DA) neurons projecting from the ventral tegmental area (VTA) to inter alia the nucleus accumbens (NAC), the central nucleus of amygdala and the medial prefrontal cortex (mPFC). The mesolimbocortical dopaminergic system has been shown to play an important role in mediating the reinforcing effect of natural rewards as well as that of various drugs of abuse including nicotine [12,14,30,63]. Thus, lesioning of the mesolimbic DA neurons attenuates nicotine self-administration as well as the locomotor stimulant effect of systemic nicotine in rats [9,11], and systemic nicotine increases DA release, synthesis and metabolism in the NAC and the mPFC [21,27,39,41]. Furthermore, in vivo electrophysiological studies have shown that systemic nicotine increases firing and specifically burst activity, i.e. rapid sequences of spikes with short inter-spike time intervals, in midbrain DA neurons [17,19]. This is of particular interest because DA neurons have been shown to influence their target areas by changing their firing pattern from single spike firing to burst firing, even without changing their...
average activity level [3]. Burst stimulation of midbrain DA neurons results in a marked increase in DA release in the nerve terminal regions [17], and in the expression of specific genes, such as the immediate early gene c-fos, in postsynaptic neurons [7,8]. Such transient changes of impulse activity normally occur in association with basic attentional and motivational processes, such as the response to sensory rewarding stimuli, and apparently serve to initiate goal-oriented behaviors

Chronic nicotine administration has been found to result in a pronounced increase in DA metabolism selectively in the prefrontal cortex [61]. In this regard, we have recently shown that chronic, intermittent administration of nicotine in rats produces sensitization of nicotine’s locomotor stimulatory action that is associated with an increase in: (i) responsiveness of VTA-DA neurons to nicotine; (ii) nicotine-induced DA release selectively in the mPFC; and (iii) nicotine-induced c-fos expression in the mPFC that involves D1-DA receptor activation [41,42]. In a follow-up study, we have recently found a selective increase in mRNAs for the immediate early genes c-fos and nerve growth factor-inducible clone A (NGFI-A) in the mPFC of rats treated chronically with nicotine, finding indicating that nicotine dependence is indeed associated with an enhanced neuronal activity in the mPFC [43]. These effects of nicotine on the mesolimbocortical DA pathways may be particularly important for the excessive tobacco-smoking clinically seen in schizophrenic patients [16,26,33]. These patients frequently display hypofrontality, i.e. a reduced functional activity in the prefrontal cortex, which correlates with negative symptoms of schizophrenia, such as lack of goal-oriented behaviors, flattening of affect and emotional indifference to normally rewarding stimuli [28]. The prefrontal cortex provides a direct input to the VTA-DA cells regulating phasic activity, i.e. essentially burst firing, but not tonic activity in these neurons through glutamatergic pathways [4,6,20,35]. Accordingly, impaired functional activity in the prefrontal cortex in the rat has been found to decrease burst firing in VTA-DA neurons and associated DA release in the NAC [36,59]. Thus, regular nicotine intake in hypofrontal individuals may reflect an attempt at self-medication. It is noteworthy that nicotine restored burst activity of DA cells in the VTA of experimentally hypofrontal animals [60]. Furthermore, we have recently shown that nicotine withdrawal reactions, precipitated by the nicotinic receptor antagonist mecamylamine in animals receiving chronic nicotine treatment, are accompanied by marked decreases in DA output in the NAC and the central nucleus of amygdala [24,47]. These findings may have bearing on motivational deficits and on symptoms, such as anxiety, distress and dysthymia, which are frequently associated with nicotine abstinence in humans [16]. Taken together, these results support the notion that the mesolimbocortical dopaminergic pathways play a protagonistic role in nicotine’s reinforcing and dependence-producing actions in association with symptoms often encountered in major neuropsychiatric disorders.

2. Nicotinic receptors in the VTA determine nicotine’s stimulatory effect on mesolimbocortical dopaminergic function

Given that nicotinic receptors are localized both in the somatodendritic and the nerve terminal regions of the mesolimbocortical dopaminergic projections, and that systemic or local administration of nicotine increases DA release in the NAC, we examined in a series of behavioral, biochemical and histochemical experiments the role of nicotinic receptors in the VTA or the NAC in the behavior-, DA- and c-fos-stimulatory actions of nicotine. Interestingly, the systemic nicotine-induced increase in DA release in the NAC was blocked by mecamylamine administered locally in the somatodendritic region of the mesolimbocortical DA neurons (Table 1), whereas mecamylamine administered locally in the NAC was without effect [39]. These results are in accordance with those of Reavill and Stolerman [50] and Leikola-Pelho and Jackson [31], which indicate that nicotine exerts a more pronounced effect on locomotor activity when it is injected in the cell body area of the mesolimbocortical DA neurons than when it is injected into the terminal region, e.g. the NAC. Moreover, Museo and Wise [37] and Panagis et al. [46] have shown a gradual increase, i.e. sensitization, of the locomotor stimulating response following repeated ventral tegmental injections of the nicotinic receptor agonist cytisine or nicotine itself, respectively. In the latter study, we also demonstrated that intrategmental injections of nicotine increase Fos-like immunoactivity in the NAC in a similar manner to that obtained after systemic nicotine injections [42,51]. In general, it appears that local administration of nicotine in the VTA produces effects on dopamine release and c-fos expression in the NAC that are comparable to those elicited after systemic administration of nicotine (Table 1) [39,40,52,55]. More recently, we provided evidence that intrategmental infusion of mecamylamine prevents the systemic nicotine-induced c-fos expression in the NAC (Table 1) [55]. Also, Corrigall et al. [10] reported that microinfusion of the nicotinic receptor antagonist dihydro-β-erythroidine into the VTA, but not into the NAC, decreases nicotine self-administration. Moreover, application of cytisine into the VTA can produce a conditioned place preference [38]. These findings...
indicate that nicotine primarily acts within the ventral tegmentum to initiate processes, which are critical for the behaviorally activating and reinforcing properties of the drug. Accordingly, local infusion of nicotine in the cell body region or the terminal region of the mesolimbocortical dopaminergic neurons elicits DA release in the NAC of equal magnitude, but only the response to nicotine applied in the terminal region showed rapid desensitization [39,40]. The more long-lasting effect of nicotine administered in the VTA on DA release in the NAC was similar to the effect of systemic nicotine, suggesting that nicotinic receptors in this region are probably more important than those located in the NAC for mediating the effect of systemically administered nicotine on accumbal DA release. In this respect, we have also provided evidence that nicotine withdrawal and associated decreases in the output of DA and its metabolites in the NAC can be elicited with both systemic and intrategmental, but not intraccumbal (B.E. Hildebrand, personal communication), injections of mecamylamine (Table 2) [23–25], a finding that emphasizes the importance of nicotinic receptors in the VTA for nicotine’s dependence-producing properties. Excitatory amino acid (EAA) inputs are of fundamental importance for the regulation of firing pattern of VTA-DA neurons as well as DA release from the nerve terminals (see above). Therefore, we sought to investigate whether N-methyl-D-aspartate (NMDA) receptors in the VTA play a role in the stimulatory actions of nicotine on DA release and c-fos expression in the NAC. Indeed, we found that intrategmental infusion of the NMDA receptor antagonist AP-5 greatly diminishes nicotine’s enhancing action on DA release and c-fos expression in the NAC (Table 1) [52,55], indicating that the nicotine-induced excitation of the mesolimbocortical dopaminergic system involves concomitant NMDA receptor activation in the VTA probably through an increase in EAA concentrations. This notion is further supported by our recent data showing an increase in extracellular concentrations of glutamate and aspartate in response to systemic or intrategmental administration of nicotine [53]. Our results confirm and extend those previously obtained by Shoaib et al. [58] and Kiba and Jayaraman [29], who have shown that blockade of NMDA receptors attenuates nicotine’s locomotor stimulant action, prevents sensitization of nicotine-induced DA release in the NAC, and reduces postsynaptic responses to systemic nicotine in the rat forebrain, respectively. Taken together, the results point to the significance of VTA nicotinic receptors for the reinforcing and dependence-producing properties of nicotine, implicating NMDA receptors within the VTA as well.

Table 1
Effects of nicotine administered alone or in the presence of mecamylamine or AP-5 in the VTA on dopamine output measured by microdialysis and on the number of cells stained positive for Fos-like immunoreactivity in the nucleus accumbens

<table>
<thead>
<tr>
<th>Treatment</th>
<th>(A) Dopamine output (% of baseline)</th>
<th>(B) Fos-like immunoreactivity (number of cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (1 ml/kg, s.c.)</td>
<td>99 ± 3</td>
<td>2.8 ± 0.9</td>
</tr>
<tr>
<td>Nicotine (0.5 mg/kg, s.c.)</td>
<td>158 ± 5***</td>
<td>13.5 ± 3.6**</td>
</tr>
<tr>
<td>Mecamylamine (0.1 mM, in VTA) + Nicotine (0.5 mg/kg, s.c.)</td>
<td>105 ± 6+</td>
<td>5.0 ± 1.1+</td>
</tr>
<tr>
<td>AP-5 (1.0 mM, in VTA) + Nicotine (0.5 mg/kg, s.c.)</td>
<td>118 ± 5++</td>
<td>5.8 ± 2.5++</td>
</tr>
<tr>
<td>Nicotine (1.0 mM, in VTA)</td>
<td>128 ± 4*</td>
<td>9.2 ± 1.3**</td>
</tr>
</tbody>
</table>

* The infusion of mecamylamine or AP-5, via a microdialysis probe implanted in the VTA, started 40 min before injection of nicotine and lasted throughout the experiment (160 and 120 min for dopamine output and Fos-like immunoreactivity measurements, respectively). Dopamine output data from the nucleus accumbens are presented as mean (± S.E.M., n = 5–8 in all groups) overall change over 160 or 80 min period after systemic injection of saline or nicotine, or after infusion of nicotine through a microdialysis probe in the VTA, respectively. Baseline (100%) was defined as the average of four 20-min pretreatment (i.e. before administration of saline, nicotine, mecamylamine or AP-5) values. Fos-like immunoreactivity data are presented as mean (± S.E.M., n = 5–8 in all groups) number of positive nuclei counted in the nucleus accumbens ipsilaterally to the microdialysis probe that was implanted in the VTA and through which mecamylamine, AP-5 or nicotine were infused.

* P < 0.05 compared to the overall dopamine output over 80 min before the start of nicotine infusion (for further details about the methodology, see Refs. [39,40,52,55]).

** P < 0.01 compared to the effects of saline. In the experiment with local infusion of nicotine in the VTA, P < 0.01 compared to the control group perfused with ordinary perfusion solution devoid of nicotine.

*** P < 0.001 compared to the effects of saline.

† P < 0.05 compared to the effects of nicotine alone. Infusion of neither mecamylamine nor AP-5 in the VTA had any significant effect on dopamine output or Fos-like immunoreactivity in the nucleus accumbens.

++, + P < 0.001 compared to the effects of nicotine alone. Infusion of neither mecamylamine nor AP-5 in the VTA had any significant effect on dopamine output or Fos-like immunoreactivity in the nucleus accumbens.
Table 2
Effects of mecamylamine or MLA on the output of dopamine and its metabolites, DOPAC and HVA, from the nucleus accumbens measured by microdialysis in control animals and in animals receiving chronically nicotine

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dopamine output (% of baseline)</th>
<th>DOPAC output (% of baseline)</th>
<th>HVA output (% of baseline)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>Nicotine</td>
<td>Sham</td>
</tr>
<tr>
<td>Mecamylamine (1 mg/kg, s.c.)</td>
<td>108 ± 5</td>
<td>87 ± 4*</td>
<td>102 ± 3</td>
</tr>
<tr>
<td>Mecamylamine in VTA (9 μg/0.5 μl)</td>
<td>103 ± 4</td>
<td>84 ± 5*</td>
<td>102 ± 4</td>
</tr>
<tr>
<td>MLA in VTA (3 μg/0.5 μl)</td>
<td>106 ± 3</td>
<td>78 ± 5**</td>
<td>105 ± 3</td>
</tr>
</tbody>
</table>

* Dopamine, DOPAC and HVA output data from the nucleus accumbens are presented as mean (± S.E.M., n = 6–12 in all groups) overall change over 180 min period after systemic injection of mecamylamine, or after unilateral injection of mecamylamine or MLA in the ventral tegmental area (VTA). Nicotine was administered via subcutaneously implanted Alzet osmotic minipumps, whereas control animals carried empty minipumps or minipumps containing saline (Sham). Injection of saline either systemically or in the VTA in control animals or in animals treated chronically with nicotine did not significantly affect the output of dopamine and its metabolites from the nucleus accumbens. Baseline was defined as the average of two 15-min pretreatment (i.e. before administration of mecamylamine or MLA). Basal values of dopamine, DOPAC and HVA were not significantly affected by chronic nicotine treatment (for further details about the methodology, see Refs. [24,24,44]).

* P < 0.05 compared to the effects in control animals.

** P < 0.001 compared to the effects in control animals.

3. Role of α7 nicotinic receptors in the ventral tegmental in nicotine’s stimulatory effects on mesolimbocortical dopaminergic function

Nicotine acetylcholine receptors are members of the superfamily of ligand-gated ion channels, including the NMDA, AMPA/kainate, GABAA, 5-HT3 and glycine receptor families. The neuronal nicotinic receptor family is subdivided into receptors with high and low affinity for nicotine. The high-affinity nicotinic receptors, containing α subunits 2–5, require a β subunit for function, whereas the low-affinity nicotinic receptors do not. The low-affinity subunits, including α 7–9, also bind the snake toxin, α-bungarotoxin (α-BTX). Of these α-BTX-binding subunits, only the α7 is found in the mammalian brain [62,64].

Patients suffering from schizophrenia typically exhibit deficits in sensory processing, e.g. they are unable to filter out or gate repetitive auditory stimuli [1]. Intake of nicotine is able to transiently reverse this deficit in non-gating, schizophrenic patients, suggesting a deficiency in cholinergic transmission through the nicotinic receptors in this disorder [32]. In an animal model of auditory gating deficit, selective antagonists of α7 nicotinic receptors or intracerebroventricular injections of antisense oligonucleotides complementary to the translation start site of the α7 receptor mRNA, that result in a reduction of their number by 40% in hippocampus, block physiological gating [32]. In addition, expression of the α7 nicotinic receptor is decreased in hippocampal brain tissue dissected post-mortem from schizophrenic patients [32]. Moreover, a neurophysiological defect (i.e. P50 auditory sensory deficit) in schizophrenia is genetically linked to the locus of the α7 nicotinic receptor gene on chromosome 15 [15]. These findings indicate that a deficit in the function of α7 nicotinic receptors in the hippocampus of schizophrenic patients may underlie some of the core symptoms in schizophrenia. Nicotinic receptors containing the α7 subunits are expressed on presynaptic terminals; stimulation of these receptors by nicotine results in an increase in the influx of Ca2+ in presynaptic terminals leading to an enhanced glutamatergic synaptic transmission in certain brain structures, such as the habenula and the hippocampus [18,34].

Previous studies suggest that the α7 nicotinic receptors subserve an indirect, presynaptic action in the regulation of neurotransmitter release. Therefore, we investigated whether these receptors have any place in the sequence of events involving nicotinic and NMDA receptors within the VTA, as described above. As previously shown by Dominguez del Toro et al. [13], α7 nicotinic receptors were indeed localized in the VTA [54]. Importantly, we showed that infusion of the selective α7 nicotinic receptor antagonist methyllycaconitine (MLA) in the VTA dose-dependently diminishes the systemic nicotine-induced DA release in the NAC [54]. Additionally, we showed that ibotenic acid lesions of the mPFC resulted in a decrease in the number of neurons exhibiting α7 nicotinic receptor immunoreactivity in the VTA and decreased α-BTX binding, i.e. α7 receptor content, in the VTA by about 30% [53]. These observations suggest that at least a part of the α7 nicotinic receptor content in the VTA is localized presynaptically on corticotegmental afferents, which probably utilize EAAs as their neurotransmitter (see above). Tentatively, stimulation of the α7 nicotinic receptors in the VTA by nicotine results in an enhanced release of EAAs and, consequently, in stimulation of VTA-DA neuronal activity via NMDA receptors and augmentation of DA release in the NAC. In support of this hypothesis, we found that MLA infused locally in the
VTA diminished the systemic nicotine-induced increase in glutamate and aspartate concentrations in this brain region [53]. In follow-up studies we found that a nicotine withdrawal reaction and concurrent decreases in the output of DA and its metabolites in the NAC can be elicited by intrategmental injections of MLA in chronically nicotine-treated rats (Table 2) [44]. Recently, we also found that the enhancing action of nicotine on the reinforcing efficacy of intracranial self-stimulation in the medial forebrain bundle, that is indicative of an increase in brain stimulation reward, is reversed by intrategmental injections of MLA [45]. These findings suggest that \( \alpha 7 \) nicotinic receptors in the VTA play a pivotal role in nicotine’s stimulatory effect on mesolimbocortical dopaminergic function and consequently in its reinforcing and dependence-producing properties. In general, \( \alpha 7 \) nicotinic receptors in the VTA and the cholinergic projection from the pedunculopontine and laterodorsal tegmental nuclei appear to represent essential components of the neural circuit linked to the mesolimbocortical DA reward system [65]. This hypothesis is supported by the findings that intrategmental injections of MLA diminished the stimulatory action of food reward on DA release in the NAC [52] and reversed the cocaine-induced increase in brain stimulation reward [45].

4. Significance and concluding remarks

Based on the data reviewed above, we hypothesize that nicotine or reward-related enhanced concentrations of acetylcholine within the VTA stimulate \( \alpha 7 \) nicotinic receptors localized on glutamatergic afferents from the mPFC to the VTA, which, in turn, results in an increase in glutamate release, stimulation of NMDA receptors on VTA-DA neurons, increase in VTA-DA neuronal activity, DA release in the nerve terminal regions, and an increase in c-fos expression therein via activation of D1-DA receptors. Alternatively, \( \alpha 7 \) nicotinic receptors appear to be also found on dopamine neurons within the VTA, thereby regulating a nicotine-activated current [5,49]. Evidence has also been provided that nicotinic receptors other than the \( \alpha 7 \) in the VTA are involved in nicotine’s stimulatory effects on dopaminergic function and related behavioral responses [48,49]. At any rate, a cascade of neural events related to \( \alpha 7 \) nicotinic receptor stimulation within the VTA seems to be, at least in part, implicated in nicotine-induced alterations in behavioral, electrophysiological, biochemical and histochemical responses involving the mesolimbocortical dopaminergic projections and consequently in nicotine’s dependence liability. To this end, it is tempting to speculate that a deficiency in the \( \alpha 7 \) nicotinic receptor function in the VTA may also be responsible for impairments in motivational responding and cognitive performance typically seen in neuropsychiatric disorders, such as schizophrenia. In this far from complete picture some detail is added by the recent experimental and clinical findings showing that the non-competitive NMDA receptor antagonist MK-801, a psychotomimetic compound that also distorts the function of VTA-DA neurons [36], acts as a non-stereoselective antagonist at the human \( \alpha 7 \) nicotinic receptor [2], and that a decreased protein level of the \( \alpha 7 \) nicotinic receptor subunit has been detected in the frontal cortex of schizophrenic patients [22].

The scientific information presented here may add to our understanding of nicotine dependence in general, particularly by providing a neurobiological basis for the prevalence of increased smoking in schizophrenia. Hopefully, this knowledge will also produce clues as to which pharmacological means other than nicotine we might utilize to treat nicotine dependence and, thus, assist smoking cessation in society. Finally, given the close association in the clinic between nicotine addiction and schizophrenia, the possibility that a nicotinic receptor ligand, e.g. an \( \alpha 7 \) nicotinic receptor agonist, may be useful in the treatment of at least some symptoms in schizophrenia should definitely be considered.

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