ACUTE AND CHRONIC EFFECTS OF NICOTINE ON GABA NEURONS IN THE VENTRAL TEGMENTAL AREA


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INTRODUCTION

Nicoine’s (NIC) rewarding and addictive properties appear to be mediated by dopamine neurons in the midbrain ventral tegmental area (VTA) that project to limbic structures (Figure 1). GABAergic and glutamatergic synaptic inputs to VTA dopamine neurons are mediated by different NIC acetylcholine receptor (nAChR) subtypes with distinct desensitization properties. Acetylcholine inputs to the VTA originate in the habenula and project via the fasciculus retroflexus (FR) as well as pontine tegmental nucleus (PPT). Nicotine can enhance glutamatergic (GLUergic) transmission while the nAChRs on VTA neurons are antagonized, thus shifting the balance of synaptic inputs to excitation. This desensitization silences endogenous cholinergic drive to the GABAergic inputs which ultimately dishabitates DA neurons. This coordinated disinhibition and enhanced excitation likely contributes to prolonged increases in DA release and ultimately behavioral reinforcement.

RESULTS

In Vivo Studies: Effects of Systemic Nicotine Exposure on VTA GABA Neuron Firing Rate in Naïve Mice

Figure 4: Effects of Repeated Nicotine exposure on VTA GABA Neuron Firing Rate. (A) A VTA GABA neuron, with a baseline firing rate of 2000 Hz, experienced an acute NIC (2 mg/kg) injection that was followed by subsequent injections in a repeated daily dosing paradigm. NIC administration of NIC (2 mg/kg) markedly increased the firing rate of this neuron. (B) A GABA neuron that did not receive NIC administration of NIC (2 mg/kg) showed no increase in firing rate. NIC administration of NIC (2 mg/kg) significantly reduced NIC acetylcholine activation of this neuron. (C) A GABA neuron receiving NIC administration of NIC (2 mg/kg) showed no significant increase in NIC acetylcholine activation of firing rate with repeated injections.

In Vitro Studies: Local Nicotine Effects on VTA GABA Neuron Activity in Naïve Mice

Figure 2: Effects of local microinhospital application of NIC on VTA GABA neuron firing rate in naïve mice with microinhospital applications of NIC (1 mg/kg) that were administered to VTA GABA neurons in the presence of glutamatergic transmission. We tested the effects of local application of NIC on the activity of VTA GABA neurons. Microinhospital application of NIC (1 mg/kg) resulted in increased excitation effects on VTA GABA neuron firing rate. Nicotine activation of VTA GABA neurons was increased with application of NIC (1 mg/kg). These results indicate that NIC acetylcholine activation of firing rate in VTA GABA neurons is increased with repeated applications of NIC (1 mg/kg).

HYPOTHESIS

The addictive properties of nicotine are mediated by long-term adaptations in μδGABAergic nAChRs in the VTA that regulate dopamine neurotransmission in the mesolimbic reward system.

METHODS

Animal care: Male CD-1 naïve mice for GAD GFP were obtained from Taconics/LaJolla (Crl:CD-1). Acute experiments were conducted between 9:00 AM and 4:00 PM. Brains were rapid frozen on dry ice. Brains containing GAD GFP-expressing VTA neurons were dissected from naïve mice. Animal care was performed in accordance with the National Institutes of Health’s Guidelines for Laboratory Animal Care. The experimental procedures were approved separately by a Institutional Animal Care and Use Committee. All efforts were made to minimize suffering and avoid pain.

RESULTS

In Vivo Studies: Chronic Nicotine Effects GABA Neuron Firing Rate in Response to Acute Nicotine

Figure 9: Molecular effects of chronic NIC on acute activation of GABA neurons. In chronic studies, mice were treated with an i.p injection of saline (SAL) or nicotine (NIC) 5.75 mg/kg administered once daily for 14 days and studied 24 h after the last dose of SAL or NIC.

In Vivo Studies: Effects of the Glutamate Antagonist APV on Nicotine Activation of VTA GABA Neurons in Naïve Mice

Figure 8: Effects of glutamatergic antagonists on NIC-induced activation of VTA GABA neurons. APV (50 μM) was co-administered with NIC to inhibit the activation of VTA GABA neurons in naïve mice. APV (50 μM) significantly reduced NIC activation of VTA GABA neurons. APV (50 μM) significantly reduced NIC activation of VTA GABA neurons.

In Vivo Studies: Acute Nicotine Effects on Excitatory Glutamatergic Synaptic Transmission to VTA GABA Neurons

Figure 6: Effects of acute nicotine and the NMDA antagonist APV on VTA GABA neuron firing rate. Acute NIC (2 mg/kg) enhances transmission of excitation to VTA GABA neurons. A representative example of NIC (2 mg/kg) enhancement of transmission of excitation to VTA GABA neurons. APV (50 μM) significantly reduced NIC activation of VTA GABA neurons.

SUMMARY AND CONCLUSIONS

Systemic administration of nicotine (NIC) markedly increased VTA GABA neuron firing rate in naïve mice with tachyphylaxis to repeated administration in vivo.

Microelectrophoretic application of NIC also markedly increased VTA GABA neuron firing rate in vivo; however, tachyphylaxis did not occur to repeated, periodic in situ administrations.

Nicotine activation of VTA GABA neurons was blocked by IP administration of the α7 NIC antagonist methyllycaconitine, but not by the unspecific noncompetitive antagonist mecamylamine.

Activation of firing rate by α7 NIC agonist JH403 with block by MLA provides further support that NIC excites VTA GABA neurons via effects on α7 nAChRs on excitatory GLUergic terminals.

The GLU antagonist APV blocked the excitatory effects of NIC on VTA GABA neurons, providing further evidence that NIC increases the firing rate of VTA GABA neurons by releasing GLU pre-synaptically to VTA GABA neurons.

Both NIC and the α7 NIC agonist choline enhanced spontaneous EPSCs to VTA GABA neurons in vitro, supporting in vivo evidence for enhancement of GLU neurotransmission by NIC.

Chronic NIC treatment (0.7 mg/kg/day for 2 weeks) did not significantly alter baseline GABA neuron firing rate, but did decrease their sensitivity to NIC.

Chronic NIC treatment does not significantly alter dopamine neuron firing rate.

Chronic NIC treatment enhances spontaneous inhibition to VTA GABA neurons. Stimulation of the α7 nAChR is partially responsible for this effect.

Chronic NIC treated mice showed de novo expression of α6 and α7 nAChR subunits, suggesting adaptation of these neurons to chronic NIC.

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