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Electrophysiological Characteristics of Cholinergic Receptors in Mesoaccumbens Neurons

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ABSTRACT

The ventral tegmental area (VTA) is located in the midbrain and contains massive dopaminergic neurons. The VTA has received considerable attention as a potentially important brain region for the action of psychoactive drugs such as nicotine. The present study was conducted to determine the characteristics of dopaminergic neurons by focusing on the relative contribution of nicotinic and muscarinergic receptors and on sensitivity to alpha-bungarotoxin in nicotinic receptors. Under the urethane anaesthesia, the activity of dopaminergic neurons was recorded in Sprague-Dawley rats. Cholinergic agents including nicotine, muscarine, methyllycaconitine, and dihydro-beta-erythroidine were ejected iontophoretically. The activity of mesoaccumbens neurons was more increased by nicotine than muscarine. Iontophoretically ejected dihydro-beta-erythroidine inhibited the excitatory effects of nicotine. In particular, methyllycaconitine more greatly inhibited the excitatory effects of nicotine in the VTA. The distribution of the neuronal subtype sensitive to alpha-bungarotoxine was relatively high compared to the insensitive subtype located in the VTA. These results suggest that midbrain dopaminergic neurons showed highly sensitive excitatory response upon iontophoretic application of nicotine and that midbrain VTA neurons reveal the distinct characteristics in terms of nicotinic receptors.

Key words: ventral tegmental area, nucleus accumbens, nicotine, alpha-bungarotoxin, cholinergic receptors

INTRODUCTION

Many dopaminergic neurons exist in the ventral tegmental area (VTA) so called A10 dopaminergic cell group (Dahlstrom and Fuxe, 1964). The VTA can be divided into ventral and dorsal parts and also called the parabrachialis pigmentosus and nucleus paranigralis. The ventral striatum is the ventral conjuction of the caudate and putamen that merges into and includes the nucleus accumbens

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(NAc) and the striatal portions of the olfactory tubercle. The principal dopaminergic projections to the NAc arise from neurons in the VTA (Nisell et al., 1994).

Nicotine can activate VTA and substantia nigra pars compacta (SNpc) neurons, by acting at nicotinic acetylcholine receptors (nAChRs) (Clarke et al., 1985; Grenhoff et al., 1986; Calabresi et al., 1989; Pidoplichko et al., 1997; Picciotto et al., 1998) and cause release of dopamine (DA) in the NAc of rats (Nisell et al., 1994, 1995; Pontieri et al., 1996). In particular, the mesolimbic DA system has been implicated as playing an important role in both the locomotion and reinforcing effects of nicotine (Corrigall and Coen 1989; Corrigall et al., 1992, 2000). In addition, dopaminergic neurons in the VTA have muscarinic receptors (Vilaro et al., 1990). The present study was conducted to determine the characteristics of mesoaccumbens dopaminergic neurons by focusing on the relative contribution of nicotine and muscarinergic receptors and on sensitivity to alpha-bungarotoxin in nicotinic receptors.

MATERIALS AND METHODS

Subjects

Fifty adult male Sprague-Dawley rats weighing 300±50 g were subjected to the microiontophoretic study. Anesthesia was induced by intraperitoneal administration of urethane (1.25 g/kg) and rats were mounted on a stereotaxic apparatus. Rectal temperature was monitored by a thermistor and maintained 36.5°C by means of an electrically heated blanket.

Electrophysiological recording from the VTA

Microelectrophoretic applications of chemicals with calibrated currents were performed using seven-barreled glass capillary pipettes (120F, WPI, Sarasota, FL, USA) pulled in two stages with a glass microelectrode puller (PE-2, Narishige, Setagaya-ku, Tokyo, Japan). Extracellular single unit recordings were made with a central recording barrel of 7-barreled microiontophoretic pipettes (Lee et al., 1991). The tips of the electrodes were broken back under microscopic control to a 8～10 um diameter. The recording barrel was filled with a 2 M NaCl solution. Six surrounding barrels were used for drug ejections and a current balance for current neutralization.

In order to identify if neural activity comes from mesoaccumbens neurons, the NAc (A–P=+3.97, M–L=1.6, D–V=7.87 below the cortical surface, angle=20°) was electrically stimulated and the evoked potentials were recorded by the electrode positioned in the VTA area.

Action potentials were screened via a differential amplifier (AM502, Tektronix, Carrollton, TX, USA) and window discriminator (121, WPI, Sarasota, FL, USA), which generated square pulses. These pulses were fed to AD/DA converter (1401 plus, CED, Cambridge, UK) and a personal computer which generated firing rate histograms with a software for electrophysiology (SpikeII, Cambridge Electronic Design, Cambridge, U.K.). The side barrels used for drug ejection were filled with the following solutions: 0.5 M nicotine, in 120 mM NaCl, pH 3.5; 5 mM muscarin, in 200 mM NaCl, pH 4; 1 mM dihydro-beta-erythroidine, in 165 mM Nacl, pH 4.5; 5 nM methyllycaconitine, in 165 mM NaCl, pH 4.5. To distinguish the nicotinic acetylcholine receptor subtype, we used the nichotinic ACh receptor antagonists, dihydro-beta-erythroidine (competitive antagonist) and methyllycaconitine (noncompetitive antagonist). Specifically, in order to determine whether the neurons activated to nicotine is sensitive or insensitive to alpha-bungarotoxin, dihydro-beta-erythroidine (insensitive: Bachem, Torrance, CA, USA) and methyllycaconitine (sensitive: Bachem, Torrance, CA, USA) were used. Presumed dopaminergic neurones within the VTA (or A10 region; A–P=–4.2～–6.4, M–L=0.2～1.6, D–V=7.6～8.6 below the cortical surface) are well established electrophysiological criteria (Grace and Bunney, 1983) including; 1) spontanteous firing rate between 5 and 90 spikes 10 s-1 (occurring sometimes in bursts); 2) triphasic or biphasic waveforms, with an initial positive deflection followed with a prominent negative phase; 3) long action potential (duration 2～4 ms); and, 4) low pitch sound when monitored by an audioamplifier.

Statistics

Firing rates and conduction velocity of VTA neurons were expressed as the mean±SE. The number of VTA neurons responsive to chemicals was counted and analysed by χ^2-test. Probability

values smaller than 0.05 were considered significant.

RESULTS

Identification of mesoaccumbens neurons

To observe the responses of VTA neurons to iontophoretically ejected drugs, it is important to classify that each neuron projects or not to the NAc. In order to classify the VTA neurons, we stimulated the NAc electrically while recording the activity of VTA neurons. Fig. 1 shows the location of VTA neurons recorded. The VTA neurons were concentrated in 4.8～5.2 mm posterior to bregma and 0.6～1.2 mm lateral to the midline. Spontaneous firing frequency of mesoaccumbense neurones was 20.60±2.30 impulse/sec.

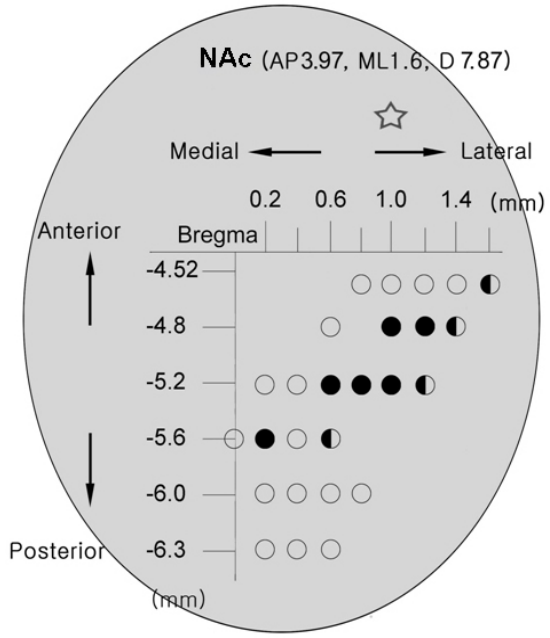

Fig. 1. The location of ventral tegmental area neurons in horizon plane. Mesoaccumbens neurons were identified by electrical stimulation of the nucleus accumbens (NAc) (☆: stimulation site, ○: unresponsive, ●: responsive, ◑: unclear).

Conduction velocity of mesoaccumbens neurons

In order to determine the conduction velocity of mesoaccumbens neurons, the latency of VTA neurons in response to electrical stimulation of the NAc was measured and divided by the distance between stimulating and recording electrodes. The conduction velocity of mesoaccumbens neurons which project from the VTA to the NAc could be readily classified into two categories showing fast and slow velocities (Fig. 2). Of 63 cells recorded in mesoaccumbens neurons, the fast neuronal conduction velocity was 2.94±0.15 m/sec and slow neuronal conduction velocity was 1.00±0.06 m/sec.

Responses of VTA neurons to iontophoretically ejected drugs

After the classification of identified mesoaccumbense neurons in the VTA by electrical stimulation, the effect of iontophoretically injected drugs was

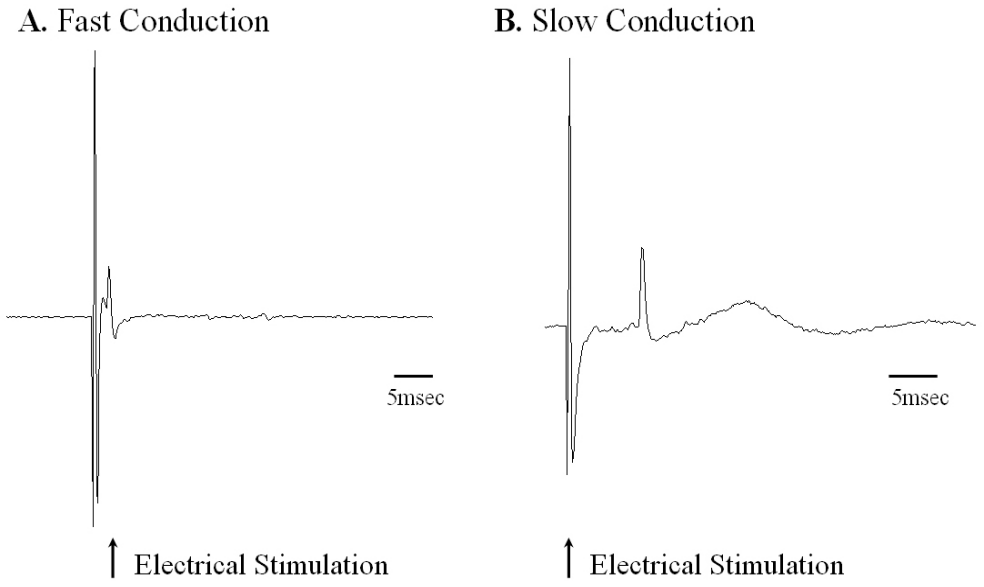

Fig. 2. Comparison of two mesoaccumbens neurons in terms of different conduction velocities. (A) Fast conduction, (B) Slow conduction.

observed. Fig. 3 shows the representative responses of VTA neurons to iontophoretically ejected chemicals. VTA neurons which project to the NAc responsed to iontophoretically ejected nicotine (Fig. 3A). Nicotine increased the activity of VTA neurons. The responses of VTA neurons to iontophoretically ejected nicotine were current-dependent. The increased responses of VTA neurons to nicotine were current-dependently inhibited by methyllycaconitine which is sensitive to alpha-bungarotoxin. Fig. 3B shows the neuronal responses to the iontophoretically ejected nicotine and dihydro-beta- erythroidine which is insensitive to alpha-bungarotoxin. Dihydro-beta-erythroidine also reduced the neuronal responses to nicotine current-dependently. Table 1 summarizes the responsiveness of VTA neurons to

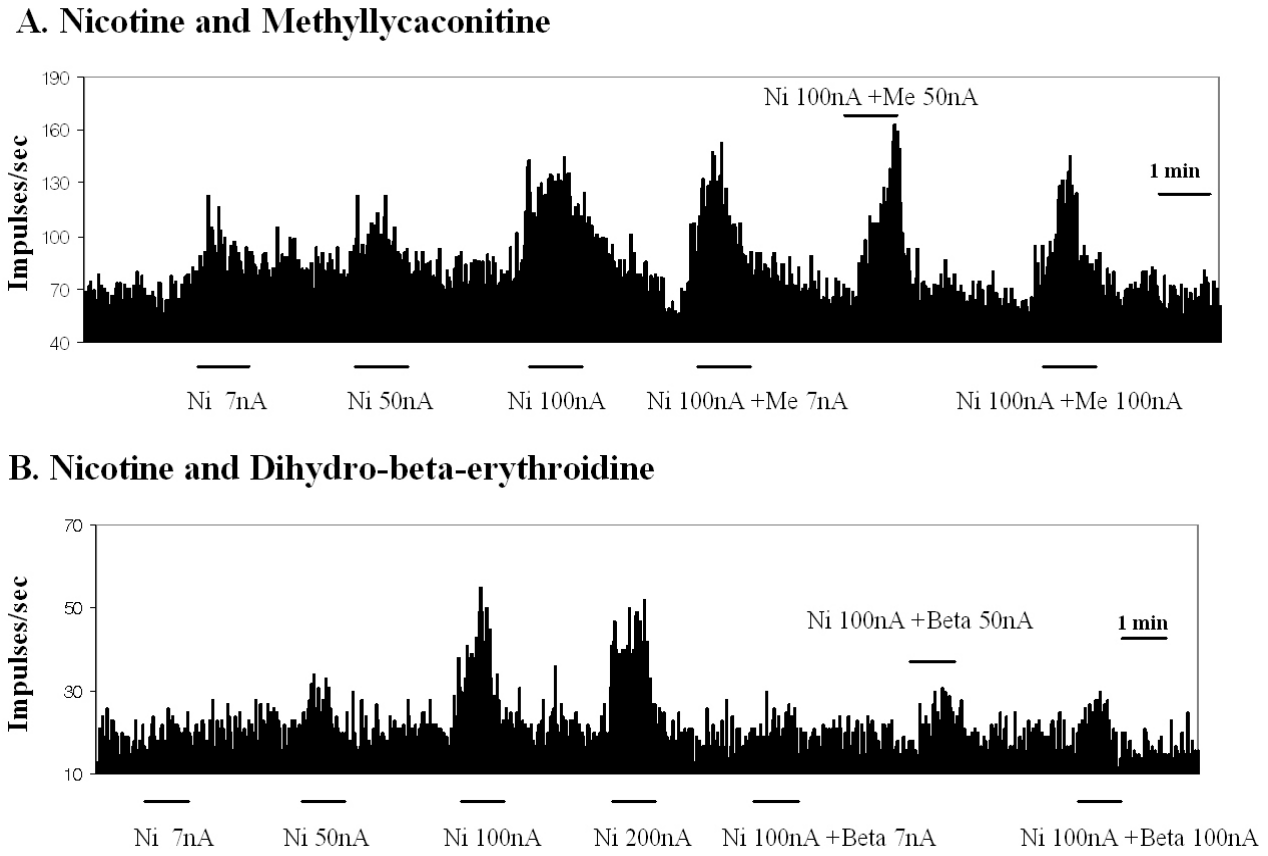

Fig. 3. The responses of VTA neurons to iontophoretically ejected chemicals. (A) Responses of VTA neurons to nicotine and methyllycaconitine. (B) Responses of VTA neurons to nicotine and dihydro-beta-erythroidine (Ni: Nicotine, Me: Methyllycaconitine, Beta: Dihydro-beta-erythroidine).

Table 1. Responses of VTA neurons to iontophoretically administered chemicals

Chemicals	Excitatory	Inhibitory	Biphasic	No effect	Total
Nicotine (NI)	14	2	13	24	53
Muscarine	4	0	2	10	16
Dihydro-beta-erythroidine (DI)	7	1	2	9	19
Methyllycarconitine (MC)	3	4	2	6	15
NI+DI	5	8	0	5	18
NI+MC	4	6	0	8	18

Excitatory: the number of neurons showing excitatory responses to each drug. Inhibitory: the number of neurons showing inhibitory responses to each drug. Biphasic: the number of neurons showing both excitatory and inhibitory responses to each drug. No effect: the number of neurons showing the no effect to each drug.

iontophoretically administered chemicals. The identified mesoaccumbens neurons showed the increased firing rate to iontophoretically ejected nicotine. The neurones were more activated by nicotine than muscarine, thus mesoaccumbens neurons were much more sensitive to nicotine than muscarine.

To determine whether the neurons activated by nicotine is sensitive or insensitive to alpha-bungarotoxin, we analysed the neuronal responses to iontophoretically ejected methyllycaconitine and dihydro-beta-erythroidine. The results suggested that the VTA neurons were not inhibited by each methyllycaconitine or dihyro-beta erythroidine in VTA neurons (χ^2=3.579, $p>.05$). When nicotine and its antagonist were concurrently ejected, the excitatory effect of nicotine was inhibited in mesoaccumbens neurons. In 8 of 18 mesoacumbense neuron (44%), the nicotinic excitatory effect was reduced by concurrently ejected dihydro-beta-erythroidine. In 6 of 18 neuron (33%), methyllycaconitine inhibited the excitatory effect of nicotine. But the difference between inhibitory effects of both dihydro-beta-erythroidine and methyllycaconitine was not statistically significant (χ^2=1.089, $p>.05$).

DISCUSSION

Systemically injected nicotine promotes the doparminergic secretion in the NAc and frontal cortex (Nisell et al., 1994). The injection of nicotine increase the activity and burst firing rate of the midbrain dopaminergic neuron (Grenhoff et al., 1986; Gonon, 1988). Our study observed the relative involvement of the nicotinic and muscarinic receptors in mesoaccumbens dopaminergic system neurons. Nicotine produces more excitatory responses than muscarin. Therefore, mesoaccumbens neurons more sensitively responded to nicotine than muscarin. It has been shown that the dopaminergic neurons in the VTA have muscarinic receptor mRNA (Vilaro et al., 1990) but the concentration of the muscarinic receptors is very low (Reisine et al., 1979; Cross and Waddington, 1980; Mash and Potter, 1986). Our results showed the current-dependent responses to iontophoretically ejected nicotine. Our data are consistent with other studies that excitatory responses to intravenously injected nicotine was dose-dependent (Armitage et al., 1968; Engberg and Svensson, 1980; Svensson and Engberg, 1980).

In order to determine whether the nicotinic receptors of VTA neurons are sensitive or insensitive to alpha-bungarotoxin, the neuronal responses were observed during iontophoretical ejection of the methyllycaconitine or dihydro-beta-erythroidin. There was no particular responses to iontophoretic ejection of dihydro-beta-erythroidine and methyllycaconitine alone in the VTA. However the nicotine-induced excitatory responses in mesoaccumbens neurons were inhibited by iontophoretically ejected nicotinic antagonists, dihydro-beta-erythroidine and methyllycaconitine. Nicotine-induced dopamine output in the nucleus accumbens was blocked by pretreatment with methllycaconitine in the VTA, indicating a role of α7 nAChRs which is the alpha-bungarotoxin binding site in this mechanism (Schilström et al., 1998). Alpha-bungarotoxin inhibits the nicotine activity in cerebellar inhibitory interneurons, but not showed specific selective inhibitory effect to nicotine in Purkinje neurons (Graza et al., 1987). Also alpha-bungarotoxin could not inhibit nicotinic and acetylcholinergic activity to various ganglionic preparation (Brown and Fumagalli, 1977; Bursztajn and Gershon, 1977). These results imply that the nicotinic receptor subtypes are different depending on the site of action. Therefore, the nicotinic receptors of dopaminergic neurons in the midbrain may have different characteristics compared to the nicotinic receptors of the other brain area neurons.

The VTA provides the main dopaminergic projections to the ventral stiatum, the prefrontal cortex, and limbic areas, and the VTA participates in reinforcement and associative learning processes. In addition, VTA projection areas have been associated with drug addiction, including nicotine (Dani and De Biasi, 2001; Dani et al., 2001). The most robust is tobacco use, particularly smoking, which is sometimes associated with a decreased incidence in neurological diseases (Checkoway and Nelson 1999; Gorell et al., 1999; Allam et al., 2004; Quik, 2004). This relationship is very consistent and time- and dose-dependent. Accumulating studies suggest that nicotine may be a candidate that mediates this apparent neuroprotection (O'Neill et al., 2002; Quik, 2004). Schizophrenics display a very high incidence of smoking, whereas nicotine is proposed to

normalize a sensory gating deficit found in shizophrenics (Stevens et al., 1998).

Therefore, nicotine and nicotinic agents may have a useful effect for various diseases such as Alzheimer's disease, Parkinson's disease, schizophrenia, and attention deficit hyperactivity disorder. These effects may be mediated by brain dopaminergic system. Thus, the further systematic study would be needed for the investigation of the effects of nicotine on brain dopaminergic system. The development of nicotine delivery system such as local injection or skin patch may reduce the misuse of nicotine and the risk of health problem and could suggest more effective treatment with reduction of the side effect of nicotine by systemic injection.

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