Excitation of Rat Striatal Large Neurons by Dopamine and/or Glutamate Released From Nerve Terminals via Presynaptic Nicotinic Receptor (\(\alpha_4\beta_2\) Type) Stimulation

Hiroaki Matsubayashi, Taku Amano, Hiroko Amano and Masashi Sasa*

Department of Pharmacology, Hiroshima University School of Medicine, 1-2-3 Kasumi, Minami-Ku, Hiroshima 734-8552, Japan

Received November 9, 2000 Accepted May 21, 2001

ABSTRACT—Previous in vivo experiments using rats anesthetized with chloral hydrate have revealed that nicotine applied iontophoretically increased firing of striatal neurons receiving excitatory dopaminergic input from the substantia nigra, and nicotine-induced firing was inhibited by domperidone, a dopamine \(D_2\) antagonist. The results suggest that nicotine increases release of dopamine from the terminals of dopaminergic neurons. Therefore, we performed the present patch clamp study using slice and acutely dissociated preparations of the rat striatum to elucidate the mechanisms underlying the nicotine-induced excitation of striatal neurons. Application of nicotine (100 \(\mu\)M) to large striatal neurons in slice preparations did not produce any effect on the resting membrane potential, but did increase the frequency of miniature postsynaptic potentials (mpps) and action potentials in all 15 neurons tested. The nicotine-induced increase in mpps and action potentials were inhibited during simultaneous application of domperidone; L-glutamic acid diethyl ester hydrochloride, a non-selective glutamate receptor antagonist; and/or dihydro-\(\beta\)-erythroidine, a central nicotinic acetylcholine receptor (\(\alpha_4\beta_2\) type) antagonist. Postsynaptic current was not induced by nicotine applied by U-tube in 96% of acutely dissociated striatal neurons. The present findings suggest that nicotine mainly acts on the presynaptic nicotinic receptors in the nerve terminals to release neurotransmitters such as dopamine and/or glutamate, thereby activating the striatal large neurons.

Keywords: Patch clamp study, Striatal neuron, Nicotinic excitation, Domperidone, L-Glutamic acid diethyl ester hydrochloride

Recently, nicotinic receptors and their mRNA have been reported to distribute in some central areas such as the hippocampus, basal ganglia and cerebral cortex by histochemical and autoradiographical studies, although details on the localization and subunit composition of the receptors still remain unclear (1 – 4). In striatal nucleus, our previous in vivo experiments using rats anesthetized with chloral hydrate indicated that nicotine applied iontophoretically increased firing of striatal neurons that received excitatory dopaminergic input from the substantia nigra (SN) (5 – 8), and the nicotine-induced firing was inhibited by domperidone, a dopamine \(D_2\)-receptor antagonist (9). The results suggest that nicotine acts on SN-derived dopaminergic nerve terminals to increase the release of dopamine from the terminals. Therefore, we performed the present patch clamp study using slice and acutely dissociated preparations of the rat striatum to elucidate the mechanisms underlying nicotine-induced excitation of striatal neurons.

MATERIALS AND METHODS

Slice patch clamp study

After decapitation of young rats (10 – 20-day-old), a block of tissue containing the corpus striatum was trimmed and kept in ice-cold artificial cerebro-spinal fluid bubbled with \(O_2\)-\(CO_2\) (95%-5%). The slices (150-\(\mu\)m-thick) including the corpus striatum were cut coronally from the block using a microslicer (DTK-1000; Dosaka, Kyoto) and were incubated at 34\(^\circ\)C for 1h before experiments. Then, the striatal slices were placed in a recording chamber perfused continuously with artificial cerebro-spinal fluid at 2 ml/min. The ionic composition of the artificial cerebro-spinal fluid for dissection and perfusion was as follows: 113 mM NaCl, 3 mM KCl, 1 mM NaH\(2\)PO\(4\), 25 mM NaHCO\(3\), 11 mM glucose, 2 mM CaCl\(2\), 1 mM MgCl\(2\). The pH of the solution was 7.4 when bubbled with \(O_2\)-\(CO_2\)
KCl, 1 mM CaCl\(_2\) following ionic composition: 130 mM K-gluconate, 10 mM 3 and 7 M/\(\text{Gb}_0\) from borosilicate capillary glass (o.d. 1.5 mm; Clark, Pangbourne, Reading, England) and had resistances between 3 and 7 M\(\Omega\) when filled with internal solution of the following ionic composition: 130 mM K-gluconate, 10 mM KCl, 1 mM CaCl\(_2\), 2 mM MgCl\(_2\), 10 mM HEPES, 10 mM EGTA (pH adjusted to 7.4 with KOH). The signals of membrane potentials were monitored on an oscilloscope (VC-11; Nikoh Kodhen, Tokyo) and recorded directly on a Thermal Array Recorder (RTA-1100, Nikoh Kodhen) and sampled at 1 kHz by a microcomputer using the program Axotape (Axon Instruments) or stored for off-line analysis on videocassettes after passage through a pulse code modulation device (VR-10B; Instutech Corporation, Great Neck, NY, USA). The membrane potentials were corrected for the liquid-junction potential between the pipette and artificial cerebro-spinal fluid by adjusting the pipette offset of Axopatch 200B at the beginning of the experiments.

**Patch clamp study for acutely dissociated preparations**

Using the same procedure for making slice preparations, slices (500-µm-thick) including the corpus striatum were cut coronally from the brain blocks of decapitated young rats (10–20-day-old) using a microslicer (DTK-1000). Parts of the striatum were rapidly excised into small pieces (2 × 2 mm\(^2\)) and then digested enzymatically by incubation for 30 min at 32°C in the artificial cerebro-spinal fluid containing 0.1% trypsin (type XI; Sigma, St. Louis, MO, USA) and bubbled with O\(_2\)-CO\(_2\) (95%-5%). Striatal neurons were dissociated into single cells by pipetting with a Pasteur pipette that had a fire-polished tip to reduce damage to the cells. The dissociated neurons were transferred into a recording chamber. After 15 min, the chamber was perfused continuously with the external bath solution containing tetrodotoxin (300 nM) at 2 ml/min. The ionic composition of the external solution was as follows: 165 mM NaCl, 5 mM KCl, 10 mM glucose, 2 mM CaCl\(_2\), 5 mM HEPES; the pH of the solution was adjusted to 7.3 with NaOH. Experiments were carried out at room temperature (21–25°C). The striatal neurons were classified into 3 groups by cell size, large (>25 µm in diameter) and medium (between 10 to 25 µm in diameter) under a Hoffman modulation microscope (×40, Nikon).

In the voltage clamp mode, drug-induced currents were recorded from these striatal neurons according to the standard whole cell patch clamp technique using an Axopatch 200A patch clamp system (Axon Instruments). Patch pipettes had resistances between 3 and 5 M\(\Omega\) when filled with internal solution of the following ionic composition: 80 mM CsCl, 80 mM CsF, 2 mM MgCl\(_2\), 10 mM HEPES, 10 mM Cs-EGTA (pH adjusted to 7.3 with CsOH). The series resistance of the patches was 10 to 25 M\(\Omega\) and was not compensated. Currents were filtered at 2 kHz and sampled directly by a microcomputer using the program pCLAMP (Axon Instruments).

The drugs were delivered to the neurons from a pore, 400 µm in diameter, at the apex of a U-shaped tube (“U-tube”) positioned about 50 µm directly above the neuronal soma (11–13).

**Data analyses**

The fluctuation of membrane potential was analyzed for mpps in current clamp mode and the peak amplitudes of the whole cell currents in voltage clamp mode were determined using the pCLAMP program. Amplitude histograms (bin width: 1 mV) of mpps for 30 s were constructed before and during application of nicotine. The numbers of mpps for 30 s were also measured in each neuron before and during application of nicotine. Significant difference was determined by statistical analysis of Student t-test.

**Drugs used**

Nicotine tartrate dihydrate (Nacalai Tesque, Kyoto); domperidone and dihydro-β-erythroidine HBr (DHβE) (RBI, Natick, MA, USA); L-glutamic acid diethyl ester hydrochloride (GDEE) and N-methyl-D-aspartic acid (NMDA) (Sigma); tetrodotoxin (TTX) and glycine (Wako Chemical, Osaka) were purchased from the indicated sources.

**RESULTS**

**Excitatory effect of nicotine on striatal large neurons in the slice preparations**

The large striatal cells identified under Nomarski optics were found to be 25 – 35 µm (30.3 ± 0.6 µm, n = 40) in long diameter and 1 – 2/4 – 6 visual fields (Fig. 1). The majority of the striatal cells were medium in size with a long diameter of 10 – 25 µm (Fig. 1). In the whole cell recording under current clamp mode, the resting membrane potential of striatal large neurons was ~61.1 ± 1.1 mV (n = 40). Bath application of nicotine (100 µM) increased the frequency of miniature postsynaptic potentials (mpps) and spike without affecting the resting membrane potentials (Fig. 2). The fluctuations of membrane potentials...
before (as control) and during application of nicotine were analyzed for mpps (Fig. 3). There was no difference of the peak frequency before and during application of nicotine (100 μM), although the second peak was seen in the latter: single peak was observed between 4 and 5 mV before the treatment, and second peak between 6 and 8 mV in the presence of nicotine. In the presence of nicotine (100 μM), the number of events detected for 30 s was increased compared with the control histogram (Fig. 3). In order to examine the dose-response relationship of nicotine-induced excitation, mpps bigger than 4 mV were counted for 30 s in each neuron. Application of nicotine (100 μM) into the bath significantly increased the number of mpps in all 15 neurons tested, although application of

Fig. 1. Large- and medium-sized striatal neurons in slice preparations. The striatal neurons were classified according to the cell size (large, >25 μm in diameter; medium, 10 to 25 μm in diameter) under a Nomarski modulation microscope (×40, Olympus). Arrows indicate large- and medium-sized neurons, respectively.

Fig. 2. Excitatory effects of nicotine (100 μM) on the striatal large neurons. Nicotine (100 μM) increased the frequency of mpps with spike, although it did not induce any depolarization shift of the resting membrane potential.
nicotine (1 and 10 μM) did not produce any effect on the membrane potentials and mpps in any of 10 neurons examined (Table 1): the mean number of mpps for 30 s before and during application of nicotine (100 μM) was significantly increased from 13.0 ± 1.2 (n = 35) to 70.4 ± 7.7 (n = 15).

To examine the neurotransmitters involved in nicotine-induced excitation, the effects of domperidone (1 μM), a dopamine D<sub>2</sub>-receptor antagonist; GDEE (10 μM), a non-selective glutamate-receptor antagonist; and DHβE (1 μM), a central nicotinic acetylcholine-receptor (α<sub>4</sub>β<sub>2</sub> type) antagonist were tested. Nicotine applied in the bath usually induced desensitization of the neuron: second application of nicotine for 2 min could not induce the same response as observed with the first treatment for 2 min in any 5 neurons tested, at the concentration of 100 μM. Therefore, domperidone (1 μM), GDEE (10 μM) or DHβE (1 μM) was applied in the bath simultaneously with nicotine (100 μM) to examine whether or not the antagonists inhibit the nicotine-induced effects, since the nicotine (100 μM)-induced excitation was observed in all large neurons.

Simultaneous addition of domperidone (1 μM) with nicotine (100 μM) failed to induce the excitatory responses in 6 of 11 large neurons examined (Fig. 4B), although those were not affected in the remaining 5 neurons (Table 2). The mean number of mpps for 30 s was significantly (P<0.01) reduced from 70.4 ± 7.7 (n = 15) during application of nicotine (100 μM) alone to 12.0 ± 2.2 (n = 6) during simultaneous application of domperidone (1 μM) with nicotine (100 μM) in 6 neurons. However, nicotine (100 μM) increased the mean number of mpps to 66.8 ± 3.2 (n = 5) even in the presence of domperidone in the remain-

| Dose of nicotine | 1 μM | 10 μM | 100 μM |
|------------------|------|-------|--------|
| mpps/30 s        | 13.0 ± 1.2 | 13.2 ± 2.3 | 14.6 ± 2.2 | 70.4 ± 7.7** |
| n                | 35   | 10    | 10     | 15      |

Mpps whose amplitudes were bigger than 4 mV were counted for 30 s before (as control) and during applications of nicotine, respectively. Nicotine (100 μM) significantly increased mpps of striatal neurons. The values and n represent mean ± S.E.M. and number of neurons tested, respectively. **P<0.01, significant difference from the control.
Nicotinic Excitation of Striatal Neuron

In addition, the nicotine-induced excitation was blocked by simultaneous application of GDEE (10 μM) with nicotine in 6 of 10 neurons, although the spontaneous mpps and firing were also inhibited by GDEE in the 6 neurons (Fig. 5A) (Table 2). The mean number of mpps for 30 s was also significantly (P<0.01) decreased to 3.3 ± 1.1 in the presence of GDEE (10 μM) with nicotine. However, GDEE did not affect the excitatory effects of nicotine in the remaining 4 neurons (Fig. 5B) (Table 2). The excitatory effects were not observed during simultaneous application of DHβE (1 μM) with nicotine in any 4 large neurons examined (Table 2).

**Fig. 4.** Inhibitory effect of domperidone on nicotine-induced excitation of striatal large neuron. Due to desensitization of the neurons exposed to nicotine applied in the bath for 2 min, the effects of second application of nicotine on the same neuron were dramatically reduced. Therefore, the effects of nicotine in the presence and absence of domperidone, GDEE or DHβE could be tested only once on the same neuron. Thus, in A, the typical excitatory effect of nicotine (100 μM) is shown. B shows the excitatory effect by nicotine (100 μM) was completely blocked during simultaneous application of domperidone (1 μM), D₂ antagonist, in the neuron different from that in A.

**Table 2.** The effects of nicotine on the activity of large neurons in the striatal slice preparations and those of DHβE, domperidone or GDEE on nicotine-induced excitation

|                | n  | no. of mpps/30 s |
|----------------|----|-----------------|
| Control        | 40 | 12.6 ± 0.8      |
| Nicotine 100 μM| 15 | 70.4 ± 7.7**    |
| Nicotine 100 μM+Domperidone 1 μM| 11| 66.8 ± 3.2** |
| Nicotine 100 μM+GDEE 10 μM| 10| 50.3 ± 6.0** |
| Nicotine 100 μM+DHβE 1 μM| 4 | 11.3 ± 0.9** |

Mpps whose amplitudes were bigger than 4 mV were counted for 30 s before (as control) and during applications of nicotine or nicotine plus antagonists, respectively. The values and n represent mean ± S.E.M. and number of neurons tested, respectively. Numbers in parenthesis show neurons of which mpps were unaffected (→), increased (↑) and decreased (↓) in the presence of nicotine (100 μM) and nicotine plus antagonists compared with the control. **P<0.01, significant difference from the control. "P<0.01, significant difference from the value during application of nicotine alone.

Mpps whose amplitudes were bigger than 4 mV were counted for 30 s before (as control) and during applications of nicotine or nicotine plus antagonists, respectively. The values and n represent mean ± S.E.M. and number of neurons tested, respectively. Numbers in parenthesis show neurons of which mpps were unaffected (→), increased (↑) and decreased (↓) in the presence of nicotine (100 μM) and nicotine plus antagonists compared with the control. **P<0.01, significant difference from the control. "P<0.01, significant difference from the value during application of nicotine alone.

Mpps whose amplitudes were bigger than 4 mV were counted for 30 s before (as control) and during applications of nicotine or nicotine plus antagonists, respectively. The values and n represent mean ± S.E.M. and number of neurons tested, respectively. Numbers in parenthesis show neurons of which mpps were unaffected (→), increased (↑) and decreased (↓) in the presence of nicotine (100 μM) and nicotine plus antagonists compared with the control. **P<0.01, significant difference from the control. "P<0.01, significant difference from the value during application of nicotine alone.

Nicotine-induced current of acutely dissociated striatal neurons

Dissociated striatal neurons were used to investigate whether or not nicotine-induced excitation of striatal neurons was caused by activation of postsynaptic nicotinic receptors. In the dissociated striatal cells, the large cells could be readily discriminated from the medium cells under the microscope. The mean diameters of the large and medium cells were 31.4 ± 0.9 μm (n = 14) and
**Fig. 5.** Inhibitory (A) and no (B) effects of GDEE on nicotine-induced excitation of striatal large neurons. A: The excitatory effect of nicotine (100 μM) was blocked during simultaneous application of GDEE (10 μM). B: The nicotine-induced effect was not affected in the presence of GDEE.

**Fig. 6.** Acutely dissociated striatal neurons. The striatal neurons were classified into 2 groups by cell size (large, >25 μm in diameter; medium, 10 to 25 μm in diameter) under a Hoffman modulation microscope (×40, Nikon). Arrows indicate large- and medium-sized neurons, respectively.
19.5 ± 0.5 μm (n = 13), respectively (Fig. 6). In most of the neurons, a part of the dendrites were reserved. In the voltage clamp mode at a holding potential of -60 mV, whole cell recording was performed to determine whether nicotine induced a current in the striatal large and medium-sized cells. In all 14 large neurons, NMDA (50 μM), glutamate NMDA-receptor agonist, produced the inward currents with peak amplitudes of 504 ± 84 (range 85 – 1180) pA in the presence of glycine (10 μM) and absence of Mg²⁺. Application of nicotine at concentrations up to 100 μM for 2 s did not induce any current in any of the same 13 neurons examined, although nicotine (100 μM) evoked a postsynaptic inward current in one large neuron. In addition, no current was induced by nicotine at concentrations up to 100 μM in any of 13 medium-sized neurons examined, although NMDA (50 μM) induced inward currents with peak amplitudes of 458 ± 73 (range 157 – 1165) pA in the same neurons as tested.

DISCUSSION

The present patch clamp studies using striatal slices have demonstrated that bath application of nicotine increases mpps and spikes without affecting the membrane potential. Nicotine-induced mpps and spikes were antagonized by domperidone and/or GDEE. These findings suggest that nicotine acts on nicotinic receptors located on the dopaminergic and/or glutamatergic nerve terminals to increase release of dopamine and/or glutamate. This conclusion was also supported by the findings that nicotine-induced mpps and spikes were inhibited by a nicotinic receptor α₄β₂-type antagonist, DH/Œ.

In addition, biochemical studies also revealed that nicotine facilitated the release of neurotransmitters such as dopamine and/or glutamate using striatal slices (14) and microdialysis (15, 16). On the other hand, the striatal neurons receiving dopaminergic input from SN are considered to be large aspiny neurons and could be excited by activating dopamine D₂ and/or D₃ receptors (5 – 7, 17, 18). These neurons were also shown to be excited by nicotine in our previous in vivo studies in which nicotine applied iontophoretically increased firing of striatal neurons receiving excitatory dopaminergic input from the substantia nigra, and nicotine-induced firing was inhibited by domperidone (9). This finding also supports the above conclusion that nicotine increases release of dopamine that activates the large neurons via D₃ receptors. Furthermore, nicotinic receptors have been found in the caudate nucleus (19), although the location of nicotinic receptor, either cell bodies or nerve terminals, remains unclear at present.

In the whole cell voltage clamp mode for acutely dissociated striatal neurons, nicotine applied by U-tube did not induce any postsynaptic current in 96% of neurons examined, although the postsynaptic nicotinic current was induced in only one of these 27 neurons examined. Proximal dendrites were reserved in the neurons examined, although the most distal dendrites were lost. These results suggest that there may be very rare postsynaptic nicotinic receptors in striatal neurons. Therefore, presynaptic nicotinic receptors are mainly located on the nerve terminals and activation of the receptors is suggested to increase release of dopamine and/or glutamate, thereby exciting the large neurons.

Acknowledgments

This work was carried out at and with the equipment of the Research Facilities for Laboratory Animal Sciences, Hiroshima University School of Medicine, and it was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture, Japan (No. 11670090 and No. 11770045) and Grants from the Smoking Research Foundation.

REFERENCES

1. Wevers A, Jeske A, Lobron Ch, Birtsch Ch, Heinemann S, Maelicke A, Schroeder R and Schroeder H: Cellular distribution of nicotinic acetylcholine receptor subunit mRNAs in the human cerebral cortex as revealed by non-isotopic in situ hybridization. Mol Brain Res 25, 122 – 128 (1994)
2. Schulz DW, Loring RH, Aizenman E and Zigmond RE: Autoradiographic localization of putative nicotinic receptors in the rat brain using 125I-neuronal bungarotoxin. J Neurosci 11, 287 – 297 (1991)
3. Clarke PBS, Schwartz RD, Paul SM, Pert CD and Pert A: Nicotinic binding in rat brain autoradiographic comparison of 3H-acetylcholine, 3H-nicotine, and 125I-l-bungarotoxin. J Neurosci 5, 1307 – 1315 (1985)
4. Shimohama S, Taniguchi T, Fujiwara M and Kameyama M: Biochemical characterization of the nicotinic cholinergic receptors in human brain; binding of (–)3H-nicotine. J Neurochem 45, 604 – 610 (1985)
5. Akaike A, Ohno Y, Sasa M and Takaori S: Excitatory and inhibitory effects of dopamine on neuronal activity of the caudate nucleus neurons in vitro. Brain Res 418, 262 – 272 (1987)
6. Ohno Y, Sasa M and Takaori S: Dopamine D-2 receptor-mediated excitation of caudate nucleus neurons from the substantia nigra. Life Sci 37, 1515 – 1521 (1985)
7. Todo N, Momiyama T, Amano T, Kohno Y and Sasa M: Excitation by talipexole, a dopamine D2 agonist, of caudate nucleus neurons activated by nigral stimulation. Life Sci 54, 957 – 966 (1994)
8. Matsubayashi H, Amano T, Hongjing Y, Kohno H and Sasa M: Action of intravenously administered talipexole on the rat striatal neurons receiving excitatory input from nigral dopamine neurons. Psychopharmacology 120, 369 – 375 (1995)
9. Yu H, Matsubayashi H, Amano T, Cai J-Q and Sasa M: The enhancing effects of nicotine on dopamine release in the nigro-striatal dopamine system. Brain Res 872, 223 – 226 (2000)
10. Hamill OP, Marty A, Neher E, Sakmann B and Sigworth FJ: Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflugers
11 Albuquerque EX, Costa ACS, Alkondon M, Shaw KP, Ramoa AS and Aracava Y: Functional properties of the nicotinic and glutamatergic receptors. J Recept Res 11, 603 – 625 (1991)

12 Matsubayashi H, Swanson KL and Albuquerque EX: Amanda inhibits nicotinic acetylcholine receptor function in hippocampal neurons. J Pharmacol Exp Ther 281, 834 – 844 (1997)

13 Matsubayashi H, Alkondon M, Pereira EFR, Swanson KL and Albuquerque EX: Strychnine; a potent competitive antagonist of α-bungarotoxin-sensitive nicotinic acetylcholine receptors in rat hippocampal neurons. J Pharmacol Exp Ther 284, 904 – 913 (1998)

14 Giorguieff MF, Lefloch ML, Westfall TC, Glowinski J and Besson MJ: Nicotinic effect of acetylcholine on the release of newly synthesized ³H-dopamine in rat striatal slices and cat caudate nucleus. Brain Res 106, 117 – 131 (1976)

15 Harsing LG Jr, Sershen H and Lajtha A: Dopamine efflux from striatum after chronic nicotine: evidence for autoreceptor desensitization. J Neurochem 59, 48 – 54 (1992)

16 Lapin EP, Maker HS, Sershen H, Hurd Y and Lajtha A: Dopamine-like action of nicotine; lack of tolerance and reverse tolerance. Brain Res 407, 351 – 363 (1987)

17 Fujimoto S, Sasa M and Takaori S: Inhibition from locus coeruleus of caudate neurons activated by nigral stimulation. Brain Res Bull 6, 267 – 274 (1981)

18 Sasa M, Ishihara KI, Amano T, Matsubayashi H, Momiyama T and Todo N: D2 receptor activation in distinct striatal neurons in comparison with D3 receptors. Jpn J Psychopharmacol 17, 97 – 100 (1997)

19 Clarke PBS and Pert A: Autoradiographic evidence for nicotine receptors on nigrostriatal and mesolimbic dopaminergic neurons. Brain Res 348, 355 – 358 (1985)