Direct and Indirect Actions of Dopamine on the Membrane Potential in Medium Spiny Neurons of the Mouse Neostriatum

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Yasumoto, S., E. Tanaka, G. Hattori, H. Maeda, and H. Higashi. Direct and indirect actions of dopamine on the membrane potential in medium spiny neurons of the mouse neostriatum. J Neurophysiol 87: 1234–1243, 2002; 10.1152/jn.00514.2001. Many studies have shown dopamine (DA) to have a modulatory effect on neuronal excitability, which cannot be simply classified as excitatory or inhibitory in the neostriatum. To clarify whether the responses to DA (10–30 μM) are excitatory or inhibitory in the mouse medium spiny neurons, we examined the effects of DA agonists on the synchronous potential trajectory from the resting potential to the subthreshold potential. The DA-induced potential changes, which were estimated at the subthreshold potential (approximately −60 mV), were summarized as the combination of three kinds of responses: an initial hyperpolarization lasting approximately 1 min and a slow depolarization and/or hyperpolarization lasting more than 20 min. A D1-like receptor agonist, (R)+6-chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrobromide (SKF81297, 1 μM) mainly induced the initial hyperpolarization and slow depolarization. A D2-like receptor agonist, quinpirole rather than SKF81297-depressed all the DA-induced responses except for the slow depolarization. D2-like receptor antagonist sulphiride (1 μM) depressed both the initial hyperpolarization and slow depolarization. D2-like receptor antagonist sulphiride (1 μM) depressed all the DA-induced responses except for the slow depolarization. TTX (0.5 μM) abolished all the DA-induced responses. Bicuculline (20 μM) and atropine (1 μM) abolished the DA-induced initial hyperpolarization and slow depolarization, respectively. Either dl-2-amino-5-phosphonopentanoic acid (AP5; 100 μM) or 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 20 μM) blocked both the initial hyperpolarization and slow depolarization. The application of exogenous glutamate (Glu) mimicked the initial hyperpolarization and slow depolarization. These results suggest that the initial hyperpolarization is mainly due to GABA release via the cooperative action of D1- and D2-like receptors and Glu receptors in GABAergic interneurons, whereas the slow depolarization is mediated by acetylcholine (ACH) release via the cooperative action of mainly D1-like receptors and Glu receptors in cholinergic interneurons. The potential oscillation was generated at the subthreshold level in a Ba2+--, AP5-, CNQX-, bicuculline-, and atropine-containing medium. The oscillation was generated after the addition of TTX, Co2+-, or DA. In DA agonists, quinpirole rather than SKF81297 had a more depressive effect on the potential oscillation. These results indicate that the slow hyperpolarization is due to the suppression of noninactivating Na+-Ca2+ conductances via mainly D2-like receptors in the medium spiny neurons. In conclusion, the DA actions on the medium spiny neurons show a transient inhibition by the activation of D1- and D2-like receptors in mainly GABAergic interneurons and a tonic excitation and/or inhibition by the activation of mainly D2-like receptors in cholinergic interneurons and by the activation of mainly D2-like receptors in the medium spiny neurons, respectively.

INTRODUCTION

The striatum controls a wide variety of psychomotor behaviors. It receives widespread excitatory inputs from all regions of the cortex and the thalamus and converges with extensive dopaminergic afferents from the midbrain. The critical importance of dopaminergic innervation is amply illustrated by the devastating symptoms of Parkinson’s disease, which is caused by the degeneration of dopaminergic cells in the substantia nigra pars compacta and the consequent loss of dopamine (DA) in the striatum (Albin et al. 1989; Wooten 1994). Electrophysiological studies have shown DA to modulate the neuronal responses, which cannot be simply classified as excitatory or inhibitory (cf. Calabresi et al. 2000). For instance, either an iontophoretic or bath application of DA predominantly decreases the spontaneous or current injection-elicited firing frequency of medium spiny neurons of the neostriatum both in vivo and in vitro (Calabresi et al. 1990; Johnson et al. 1983; Pacheco-Cano et al. 1996). Application of a D1-like receptor agonist or antagonist indicates that the activation of a D1-like receptor inhibits the firing of medium spiny neurons of the striatum (Akaiki et al. 1987; Calabresi et al. 1987; Hu and Wang 1988; Twery 1994; Uchimura et al. 1986; White and Wang 1986). On the other hand, the application of a D2-like receptor agonist or antagonist shows that the activation of D2-like receptor excites (Akaiki et al. 1987; Uchimura et al. 1986) or inhibits the firing of medium spiny neurons of the striatum (Hooper et al. 1997; Hu and Wang 1988; O’Donnell and Grace 1994). However, a D1-like receptor agonist induces slow subthreshold depolarization with the augmentation of L-type Ca2+-conductance and enhances the firing produced by depolarizing current pulse injection (Hernández-López et al. 1997). The medial forebrain bundle stimulation in vivo also enhances the spontaneous discharge in a subset of medium spiny neurons, and this effect is blocked by a D1-like receptor antagonist (Gonor 1997). In addition, low doses of DA facilitate glutamate (Glu)-evoked spiking, whereas high doses inhibit the spiking in vivo (Chiodo and Berger 1986; Hu and Wang 1988; Hu and White 1997; Nisenbaum et al. 1988; Shen et al. 1992). One of the obstacles for deciphering the role of

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DA in regulating the excitability of the medium spiny neurons in vitro has been due to a lack of comprehensive studies on the electrophysiological consequences of activating the D1- and D2-like receptors. The other is the specific resting membrane property of the neuron. The resting potential is approximately −90 mV which is far from the threshold (approximately −55 mV) for spike generation. The resting potential is mainly regulated by inwardly rectifying K+ currents (Jiang and North 1991). On the other hand, at the subthreshold level of approximately −60 mV, the medium spiny neurons possess both the inward rectification resulting from the activation of sustained Na+ and Ca2+ currents (Bargas et al. 1994; Calabresi et al. 1987; Cepeda et al. 1995; Chao and Alzheimer 1995; Kita et al. 1985) and the outward rectification due to slowly activating K+ currents (Nisenbaum and Wilson 1995), which may play an important role in the spike generation.

The medium spiny neurons recorded from brain slices exhibit tonic firing patterns when the membrane is depolarized by a DC current injection. In situ, however, the medium spiny neurons show synchronous firing patterns with a long plateau depolarization for several seconds (Wilson 1993). The membrane potential thus shifts between two levels, referred to as the down state and the up state (Wilson and Kawaguchi 1996). In the down state, the neurons are relatively hyperpolarized at the level of approximately −85 mV and depolarize to the up state at the subthreshold level of approximately −60 mV. The transition from down to up state is triggered by excitatory synaptic inputs.

A similar transition could be produced by the intracellular injection of depolarizing current pulses in the medium spiny neurons in vitro. This procedure obtains distinct responses to DA because the activation of DA receptors has only a slight effect on the resting membrane potential but instead regulates multiple voltage- and ligand-gated conductances (ref. Calabresi et al. 2000; Nicola et al. 2000). We therefore examined the effects of exogenous DA, a D1-like receptor agonist, R(+)-6-chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrobromide (SKF81297), and a D2-like receptor agonist, trans-(−)-4aR-4,4a,5,6,7,8,8a,9-octahydro-5-propyl-1H-pyrazolo[3,4-g]quinoline hydrochloride (quinpirole), on the trajectory between the resting potential and the subthreshold potential, which was induced by the intracellular injection of depolarizing current pulses, in medium spiny neurons.

METH O D S

All experiments were conducted in accordance with the Guiding Principles in the Care and Use of Animals in the Field of Physiological Science of the Physiological Society of Japan and had the approval of the Institutional Animal Use and Care Committee of Kurume University. Male C57BL/6 mice (6–8 wk old) were quickly decapitated under ether anesthesia, and the forebrains were removed and placed in chilled (4–6°C) Krebs solution that was aerated with 95% O2−5% CO2. The composition of the solution was (in mM) 117 NaCl, 3.6 KCl, 2.5 CaCl2, 1.2 MgCl2, 1.2 NaH2PO4, 25 NaHCO3, and 11 glucose. The forebrains sliced with a Vibratome (Oxford) at a thickness of approximately 400 μm. A single slice containing the neostriatum was placed on a nylon net in a recording chamber (volume, 500 μl) and immobilized with a titanium grid placed on the upper surface of the section. The preparation was completely submerged in the superfused medium (temperature at 35.5 ± 0.5°C, mean ± SD; flow at 5–8 ml/min).

Intracellular recordings from the dorsal striatal neurons were made using glass micropipettes filled with 2 M K acetate with 2% biocytin (resistances of 80–110 MΩ). Intracellular recordings were obtained using an amplifier (Axon Instruments, Axoclamp 2B). The membrane potential of the impaled neuron was changed by passing the current through the recording electrode using a bridge circuit.

To identify the recording neurons as medium spiny neurons, the slices used to measure electrophysiological events, were transferred to 0.1 M phosphate buffer solution with 4% paraformaldehyde buffered to pH 7.4 for biocytin staining. After overnight fixation, slices were washed with alcohol (80%) and subsequently dimethylsulfoxide (DMSO). Slices then were transferred to 0.1 M phosphate-buffered saline (NaCl, 150 mM, pH 7.0) and rinsed. The slices were pretreated with triton-X (0.05%) containing Tris buffer (pH 7.0), followed by the addition of extravidin-horse radish peroxidase conjugates (buffer: extravidin = 1.000:1). After overnight incubation with extravidin-horse radish peroxidase conjugate, the slices were reacted with diaminobenzidine (0.05%) and hydrogen peroxide (0.03%). The slices were rinsed in Tris buffer and then mounted in glycerol and examined by light microscopy.

All drugs were dissolved in Krebs solution and then were applied to the preparation by superfusion. The drug solution reached a steady-state concentration in the recording chamber in 15–20 s after switching the three-way cock. The responses to the application of DA agonists such as DA, SKF81297, or quinpirole for a period of 1 min were similar to those observed after a prolonged application (up to 3 min). We therefore chose the 1- to 2-min application to obtain a sufficient reproducibility of the response. The drugs used were SKF81297, quinpirole, and S(−)-sulpiride (all from RBI); CNQX and DL-2-amino-5-phosphono pentanoic acid (AP5, all from Tocris Neuramin); DA, γ-amino-butylic acid (GABA), sodium L-glutamate monohydrate (Glu), acetylcholine chloride (ACh), atropine sulfate monohydrate, tetrodotoxin (TTX), and dimethyl sulfoxide (DMSO, all from Wako); R(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride (SCH23390), (+)-bucine culline, biocytin, extravidin-horseradish peroxidase conjugate, and diaminobenzidine (all from Sigma); and hydrogen peroxide (from Mitsubishi Kasei).

To study the subthreshold potential oscillation, the membrane potentials were digitized at 0.33 kHz. The signals were digitally recorded for 180 s by using the Clampex 8 (Axon Instruments). The recorded signals were analyzed using the Axograph 3 (Axon Instruments). All quantitative results are expressed as the means ± SD. The number of neurons examined is given in parentheses. The paired and unpaired t-tests were used to compare the data, with P < 0.05 considered to be significant.

R E S U LT S

This study was based on the intracellular recordings from 192 medium spiny neurons in the dorsal striatum of adult mice with stable membrane potentials of more negative than −80 mV. The resting membrane potential and the apparent input resistance were −87 ± 5 mV and 42 ± 11 MΩ (n = 192), respectively. The threshold of the tetrodotoxin (TTX, 0.5 μM)-sensitive spike was −55 ± 5 mV (n = 192) when an action potential was elicited by depolarizing current pulses (0.3–0.7 nA for 25 ms every 3 s). To obtain distinct responses induced by exogenous DA, the membrane potential was depolarized to the subthreshold level (approximately −60 mV) by injecting depolarizing current pulses (intensity, 0.3–0.7 nA for 1.5 s every 3 s) through the recording electrodes. This potential trajectory induced by the depolarizing current pulses mimics synchronous potential changes from a down state (approxi-
mately −85 mV) to an up state (approximately −60 mV) in the in vivo neostriatal medium spiny neurons (Wilson 1993).

Changes in the membrane potentials induced by exogenous DA and DA agonists in medium spiny neurons

The responses to application of exogenous DA (10−30 µM, 1-min application), which were estimated at the subthreshold potential (approximately −60 mV), varied from cells to cells. These potential changes consisted of the combination of three kinds of responses. Figure 1A shows two typical responses. One is a biphasic response consisting of an initial hyperpolarization and a subsequent depolarization (slow depolarization; top). The other is a triphasic response consisting of an initial hyperpolarization and a subsequent slow depolarization, which was followed by a prolonged hyperpolarization (slow hyperpolarization; bottom). The initial and slow hyperpolarization were associated with an increase in the membrane conductances, whereas slow depolarization was accompanied by a decrease in the membrane conductance.

Table 1 is a summary of various potential changes induced by DA. The initial hyperpolarization and subsequent slow depolarization were most frequently observed. The second frequently observed response was the slow depolarization alone. The third response was the initial hyperpolarization and the subsequent slow depolarization, which was followed by the slow hyperpolarization. The amplitude and duration of the initial hyperpolarization were −4 ± 3 mV and 46 ± 19 s (n = 23), respectively. The slow depolarization frequently triggered action potentials so that the amplitude was measured from the resting potential to the firing baseline level recorded by an x-y recorder with low-pass-filter. The amplitude of all the slow depolarization was 6 ± 3 mV (n = 34). However, the duration was quite different in the slow depolarization with or without the subsequent slow hyperpolarization: the former duration was 3.7 ± 2.4 min (n = 15) whereas the latter was more than 20 min (n = 19). The amplitude of the slow hyperpolarization was −6 ± 3 mV (n = 13). In 4 of 13 neurons tested, the slow hyperpolarization was recovered to the preapplication level approximately 20 min after washing out DA and its duration was 14.1 ± 7.8 min (n = 4). In the remaining nine neurons, the slow hyperpolarization lasted until 30 min after the onset of DA application.

Figure 1B shows concentration-response relationships of the initial hyperpolarization, slow depolarization, and slow hyperpolarization induced by DA. The responses were increased in amplitude with a concentration-dependent manner and their EC50s were 72 ± 18 (n = 14), 299 ± 90 (n = 14), and 140 ± 40 nM (n = 13), respectively. In all experiments, impaled neurons were identified whether they were medium spiny neu-

**FIG. 1.** Responses to dopamine (DA) of medium spiny neurons in mouse dorsal neostriatum. In all recordings, the membrane was depolarized from the resting potential to the subthreshold level by injection of depolarizing current pulses (intensity 0.3−0.7 nA for 1.5 s every 3 s) to obtain distinct responses induced by administration of DA (indicated by horizontal bars). A: typical potential changes induced by superfusion with DA (30 µM) for 1 min. Top: biphasic potential changes (an initial hyperpolarization and a subsequent slow depolarization). Bottom: biphasic potential changes followed by a slow hyperpolarization. B: concentration-response curves for the DA-induced potential changes. The amplitude of the DA-induced initial hyperpolarization (○), slow depolarization (●), and slow hyperpolarization (□) are plotted. Each point and vertical bar represents the mean ± SD of 14 different neurons in each group except for the slow hyperpolarization (n = 13). The concentration-response curves were fitted using the Kaleidagraph software package. C: a typical recording neuron which was stained by biocytin staining. A horizontal bar represents 50 µm.
rons or not by biocytin staining after recording any electrophysiological events. Figure 1C shows a typical medium spiny neuron. The neurons had a polygonal cell body with 17 ± 4 μm of the long axis and 12 ± 3 μm (n = 192) of the short axis, and their dendrites had immense spines.

To clarify whether a dopamine 1 (D<sub>1</sub>)-like receptor and/or dopamine 2 (D<sub>2</sub>)-like receptor mediate these responses, the effects of a D<sub>1</sub>-like receptor agonist, SKF81297 (1 μM), and a D<sub>2</sub>-like receptor agonist, quinpirole (1 μM), were examined. Figure 2A shows typical potential changes induced by SKF81297 and quinpirole. SKF81297 (1 μM) induced an initial hyperpolarization and a subsequent slow depolarization (Fig. 2A, top). On the other hand, quinpirole induced an initial hyperpolarization, a slow depolarization and a long-lasting slow hyperpolarization (Fig. 2A, bottom). These potential and conductance changes induced by the DA agonists were very similar to those induced by DA. Table 1 summarizes the various responses induced by the DA agonists. SKF81297 mainly induced a monophasic slow depolarization (32% in 22 neurons tested) and a biphasic response consisting of an initial hyperpolarization and a subsequent slow depolarization (32%). Quinpirole mainly induced either a biphasic response consist-

![Table 1: Changes in the membrane potentials induced by DA agonists](http://jn.physiology.org/)

<table>
<thead>
<tr>
<th>DA analogues</th>
<th>Initial Hyperpolarization and Slow Depolarization (%)</th>
<th>Slow Depolarization (%)</th>
<th>Initial Hyperpolarization (%)</th>
<th>Slow Hyperpolarization (%)</th>
<th>Slow Depolarization and Slow Hyperpolarization (%)</th>
<th>Slow Hyperpolarization and Slphone</th>
<th>No Response (%)</th>
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<tr>
<td>Dopamine, 30 μM</td>
<td>38 (14)</td>
<td>27 (10)</td>
<td>19 (7)</td>
<td>8 (1)</td>
<td>5 (2)</td>
<td>3 (1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>SKF81297, 1 μM</td>
<td>32 (7)</td>
<td>32 (7)</td>
<td>0 (0)</td>
<td>4.5 (1)</td>
<td>4.5 (1)</td>
<td>0 (0)</td>
<td>27 (6)</td>
</tr>
<tr>
<td>Quinpirole, 1 μM</td>
<td>14 (3)</td>
<td>9 (2)</td>
<td>27 (6)</td>
<td>0 (0)</td>
<td>27 (6)</td>
<td>14 (3)</td>
<td>9 (2)</td>
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The changes in the membrane potentials were observed at the subthreshold level of the membrane. The number of neurons is shown in parentheses.

![Fig. 2](http://jn.physiology.org/) Responses to DA agonists and inhibitory actions of both DA receptor antagonists and TTX in medium spiny neurons. **A**: typical potential changes induced by superfusion with (+)-6-chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrobromide (SKF81297; 1 μM) or trans-(-)-4aR-4,4a,5,6,7,8,9-octahydro-5-propyl-1H-pyrazolo[3,4-g]quinoline hydrochloride (quinpirole, 1 μM) for 2 min. **Top**: biphasic potential changes induced by D<sub>1</sub>-like receptor agonist, SKF81297. **Bottom**: triphasic potential changes induced by D<sub>2</sub>-like receptor agonist, quinpirole. **B**: effects of DA receptor antagonists and TTX on the DA-induced potential changes. **Top**: biphasic responses to DA after pretreatment with D<sub>1</sub>-like receptor antagonist, SCH 23390 (1 μM) for 10 min. **Middle**: biphasic responses to DA after the pretreatment with D<sub>2</sub>-like receptor antagonist, sulpiride (1 μM) for 10 min. **Bottom**: no response to DA, except for a small slow hyperpolarization, in the slice pretreated with TTX (0.5 μM) for 10 min.
Mechanisms underlying the generation of the initial hyperpolarization and the slow depolarization produced by DA

In the slices, pretreated with a selective γ-aminobutyric acid A (GABA_A) receptor antagonist, bicuculline (20 μM), the DA-induced initial hyperpolarization was markedly suppressed (n = 5; Fig. 3A). In contrast, the pretreatment with a muscarinic receptor antagonist, atropine (1 μM) abolished the DA-induced slow depolarization (n = 5; Fig. 3B). These results suggest that the initial hyperpolarization and the slow depolarization may be due to the activations of GABAergic and cholinergic interneurons, respectively, due to the application of exogenous DA.

To evaluate this possibility, the reversal potentials for the DA-induced initial hyperpolarization and the DA-induced slow depolarization were estimated by interpolating and extrapolating the values, respectively, of each of the amplitudes measured at the resting and the subthreshold levels. The estimated reversal potential for the initial hyperpolarization was −78 ± 7 mV (n = 23) and that for the slow depolarization was −92 ± 3 mV (n = 34). Figure 4 shows the responses to the exogenous GABA (5 mM) and acetylcholine (ACh, 10 mM) in the absence and presence of TTX (0.5 μM). The responses in TTX media were similar to the respective controls: the GABA-induced hyperpolarization was associated with an increase in the membrane conductance, whereas the ACh-induced depolarization was accompanied by a decrease in the conductance. In the presence of TTX, the estimated reversal potential for the GABA-induced hyperpolarization was −68 ± 6 mV (n = 5) and that for the ACh-induced depolarization was −100 ± 12 mV.

In the slices pretreated with a selective D_2-like receptor antagonist, sulpiride (1 μM), DA (10–30 μM) could not produce any response in most neurons (n = 9), but it did induce a small hyperpolarization in only one neuron shown in Fig. 2B, thus indicating that most of the responses produced by DA are TTX sensitive.

D_2-like receptors, and the slow depolarization and the slow hyperpolarization may be due to the activation of mainly D_1- and D_2-like receptors, respectively. In the slices pretreated with TTX (0.5 μM), DA (30 μM) could not produce any response in most neurons (n = 9), but it did induce a small hyperpolarization in only one neuron shown in Fig. 2B, thus indicating that most of the responses produced by DA are TTX sensitive.

FIG. 3. Effects of either bicuculline or atropine on responses to DA in medium spiny neurons. A: compared with the control response to DA (top), pretreatment of the slice with bicuculline (20 μM) for 10 min abolished the initial hyperpolarization and prolonged the slow depolarization (bottom). The traces were obtained from the same neuron. B: compared with the control response to DA (top), pretreatment of the slice with atropine (1 μM) for 10 min abolished the slow depolarization (bottom). The traces were obtained from the same neuron.

FIG. 4. Responses to GABA and acetylcholine (ACh) in the absence and presence of TTX in medium spiny neurons. A: typical responses to GABA in the absence (top) and presence of TTX (0.5 μM, bottom) in different neurons. The similar responses were observed in the absence and presence of TTX. B: typical responses to ACh in the absence (top) and presence of TTX (0.5 μM, bottom) in different neurons. The responses were similar in the absence and presence of TTX.
mV (n = 7). The reversal potential for the response to GABA was more positive than that for the initial hyperpolarization (P < 0.01, with unpaired t-test), while the reversal potential for the ACh response was more negative than that for the slow depolarization (P < 0.01, with unpaired t-test). It is possible that in addition to either the activation of GABAA or muscarinic receptors, other intrinsic currents activated by DA may be involved in the initial hyperpolarization and subsequent depolarization (see DISCUSSION). Nevertheless, DA-induced initial hyperpolarization and slow depolarization was blocked by bicuculline and atropine, respectively, thus suggesting that the activation of the GABAergic and cholinergic interneurons by DA mainly involve the generation of both the initial hyperpolarization and the slow depolarization, respectively.

Mechanisms underlying the generation of the slow hyperpolarization produced by DA

Medium spiny neurons in the striatum possess an inward-going rectification due to Na+ and Ca2+ currents at the subthreshold potential. In fact, in all neurons tested (n = 192), a strong inward rectification was observed at the subthreshold potential (Fig. 5A). This inward rectification is suppressed by either the addition of TTX (Calabresi et al. 1987) or a reduction in external Ca2+ (Bargas et al. 1994) and is augmented by the application of Ba2+ (Bargas et al. 1994). The inward rectification generates the subthreshold potential oscillation that triggers action potentials in prefrontal neurons (Tanaka et al. 1991) and nucleus accumbens neurons (Uchimura et al. 1989b). Because the DA-induced slow hyperpolarization was only observed at the subthreshold level and was blocked by TTX, DA is therefore considered to suppress the subthreshold potential oscillation.

We therefore examined the effects of DA agonists on the subthreshold potential oscillation. To generate the intrinsic subthreshold potential oscillation, Ba2+ (1 mM), a N-methyl-D-aspartate (NMDA)-type glutamate (Glu) receptor antagonist, AP5 (100 μM), an AMPA-type Glu receptor antagonist, CNQX (20 μM), bicuculline (20 μM), and atropine (1 μM) were added to the superfusing medium. Superfusion of the slices with this medium depolarized the membrane over the threshold potential, so that a hyperpolarizing DC current (approximately 0.1 nA) was continuously injected to keep the membrane potential just below the threshold level. As a result, the potential oscillation was constantly observed when the membrane potential was maintained between 61 and 65 mV. All the potential oscillations were recorded at membrane potentials between -61 and -65 mV.
reduced the power densities in the same frequency range (Fig. 6, bottom). Superfusion with Glu (10 mM) for 10 s mimicked DA-induced initial hyperpolarization and subsequent slow depolarization (control, top). After treatment of bicuculline (20 μM), Glu-induced initial hyperpolarization was abolished and unmasked the fast depolarization (bottom). B: Glu induced a fast and a subsequent slow depolarization (control, top). The Glu-induced slow depolarization was abolished after the treatment of atropine (1 μM; bottom).

membrane potential was kept at −65 ± 5 mV (n = 34), which was estimated by an x-y recorder with a low-pass-filter.

TTX (0.5 μM) or Ca2+ (2 mM) markedly suppressed the intrinsic potential oscillation and produced a hyperpolarization with the amplitude of approximately 5 mV (n = 5; Fig. 5B, inset traces). DA (30 μM), SKF81297 (1 μM), or quinpirole (1 μM) also depressed the potential oscillation and induced a small hyperpolarization (5 of 8 neurons tested for DA, 8 of 10 neurons tested for SKF81297, and 8 of 13 neurons tested for quinpirole). When the membrane potential shifted to the pre-exposure level due to a reduction in the injected hyperpolarizing DC current, the potential oscillation was still suppressed in all the neurons tested. As shown in Fig. 5, B and C, the power density spectra of the potential oscillation was obtained in the frequencies from 0.33 to 100 Hz by using the fast Fourier transform. TTX (0.5 μM) or Ca2+ (2 mM) reduced the power densities in the frequencies between 0.33 and 5 Hz (Fig. 5B).

DA (30 μM), SKF81297 (1 μM), or quinpirole (1 μM) also reduced the power densities in the same frequency range (Fig. 5C). Compared to SKF81297, DA and quinpirole markedly decreased the power densities. These results suggest that the DA-induced slow hyperpolarization is due to the suppression of the non-inactivating Na+ and Ca2+ conductances.

Possible involvement of the Glu release from the nerve terminals in the indirect responses to exogenous DA

As shown in Fig. 6, A and B, the application of exogenous Glu (10 mM) induced either a fast hyperpolarization (3 of 17 neurons tested) or a fast depolarization (14 neurons), which was followed by a slow depolarization (14 neurons). Bicuculline (20 μM) suppressed the fast hyperpolarization (Fig. 6A, top) and resutantly unmasked the fast depolarization (n = 3, Fig. 6A, bottom). Atropine (1 μM) selectively suppressed the slow depolarization (n = 5, Fig. 6B): the slow depolarization was abolished in two neurons and also was reduced by approximately 35% of the control in the other three neurons. The atropine-resistant slow depolarization was abolished by the further addition of AP5 (100 μM). Figure 7 demonstrated a typical biphasic response induced by exogenous DA in the normal medium (top). The treatment of the slices with AP5 (100 μM) completely blocked the DA-induced biphasic response, and unmasked the DA-induced slow hyperpolarization (n = 4; Fig. 7, middle). CNQX (20 μM) also blocked the DA-induced biphasic response and produced only a small amplitude of the DA-induced slow hyperpolarization (n = 3; Fig. 7, bottom). In the majority of the neurons, neither AP5 (100 μM) nor CNQX (20 μM) itself produced any potential change (n = 14). In the remaining four neurons, however, AP5 produced a depolarization of a few millivolts. These results, together with the result that the TTX completely blocked the DA-induced membrane responses, thus suggested that the synaptically released endogenous Glu probably plays a role in both the generation of the DA-induced initial hyperpolarization and the subsequent slow depolarization (see DISCUSSION).

DISCUSSION

The neostriatal medium spiny neurons are divided into two groups based on anatomical and biochemical observations: one group projects to the substantia nigra (SN) and the internal segment of the globus pallidus (GPI), whereas the other projects to the external segment of the globus pallidus (GPe) (Alexander et al. 1986; Gerfen 1992). The striato-nigral (GABA- and substance P-containing) medium spiny neurons enrich D1 receptor mRNA, while the striato-pallidal (GABA- and enkephalin-containing) medium spiny neurons enrich D2 receptor mRNA (Gerfen et al. 1990). This segregation, however, is not exclusive: the striato-pallidal neurons also contain low levels of D1 receptor mRNA and the striato-nigral neurons possess low levels of D2 receptor mRNA (Aizman 2000; Surmeier et al. 1996). D3 receptor mRNA may also be expressed at a significant level in a subgroup of DA neurons (Bordet et al. 1997). It is therefore highly possible that application of exogenous DA produces the potential change mediated by D1- and/or D2-like receptors in both the striato-nigral and -pallidal medium spiny neurons, and the present study clearly demonstrated the potential changes via D1-like receptor and/or D2-like receptor in any of the medium spiny neurons tested.

FIG. 7. Responses to DA in the absence and presence of either DL-2-amino-5-phosphonopentanoic acid (AP5) or 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) in medium spiny neurons. A: superfusion with DA induced the initial hyperpolarization and subsequent slow depolarization (control, top). After treating the slice with AP5 (100 μM) for 10 min, DA produced only a slow hyperpolarization in the same neuron (bottom). B: in a different neuron, DA produced only a slow hyperpolarization after treating the slice with CNQX (20 μM) for 10 min.
Direct action of exogenous DA on the medium spiny neurons

The DA-induced slow hyperpolarization was observed only at the subthreshold potential. The potential oscillation generated at the subthreshold level was depressed by DA, which induced a hyperpolarization similar to the slow hyperpolarization. This potential oscillation probably results from the periodic activations of both the non-inactivating Na\(^{+}\)-Ca\(^{2+}\) currents (Bargus et al. 1994; Calabresi et al. 1987) and slowly activating non-inactivating K\(^{+}\) currents (Nisenbaum and Wilson 1995) because both the currents are also only activated at the subthreshold level. The potential oscillation was generated in the medium contained Ba\(^{2+}\), AP5, CNQX, bicuculline, and atropine, thus suggesting that the contamination of spontaneous synaptic potentials in the oscillation is, if present, minimal. The DA-induced slow hyperpolarization is therefore due to the depression of the non-inactivating Na\(^{+}\)-Ca\(^{2+}\) currents in the medium spiny neurons. DA has also been reported to suppress a TTX-sensitive persistent Na\(^{+}\) conductance in rat medium spiny neurons (Cepeda et al. 1995).

The suppression of the potential oscillation by DA is not considered to be the result of an augmentation of the slowly activating non-inactivating K\(^{+}\) currents because a non-selective K\(^{+}\) channel blocker, Ba\(^{2+}\), partially depresses the slowly activating non-inactivating K\(^{+}\) currents. The subthreshold potential oscillation generated by the non-inactivating Na\(^{+}\)-Ca\(^{2+}\) conductance in the present study is comparable to the potential oscillations that are produced by non-inactivating Na\(^{+}\) conductance in the rat nucleus accumbens neurons (Uchimura et al. 1989b) and the non-inactivating Na\(^{+}\)-Ca\(^{2+}\) conductance in the guinea pig prefrontal cortical neurons (Tanaka et al. 1991). Similarly, the activation of GABA\(_B\) receptors and/or \(\mu\)-opioid receptors are not considered to be involved in the DA-induced slow hyperpolarization because Ba\(^{2+}\) may depress the K\(^{+}\) conductances via the GABA\(_B\) receptors and \(\mu\)-opioid receptors. The present results seem to correlate with the previous results in which DA was shown to be suppressed via D\(_1\) receptor the subthreshold inward rectification due to non-inactivating Na\(^{+}\)-Ca\(^{2+}\) currents in rat neostriatal neurons (Calabresi et al. 1987). In mouse medium spiny neurons, however, exogenous DA and quinpirole rather than SKF81297 more effectively produced a long-lasting slow hyperpolarization. These potencies were consistent with the inhibitory effects of these DA agonists on the subthreshold potential oscillation. Therefore the activation of D\(_2\)-like receptor plays an important role in the generation of slow hyperpolarization in mouse medium spiny neurons. As shown in Table 1, a slow hyperpolarization was induced in 35% of neurons tested by DA and in 68% of neurons by quinpirole. This difference may be due to the expression of both D\(_1\) and D\(_2\)-like receptors in the neurons tested; the DA-induced slow depolarization via D\(_1\)-like receptor is thus considered to mask the slow hyperpolarization. Voltage-clamp studies demonstrated that the activation of either D\(_1\)-like receptors (Schiffmann et al. 1995) or D\(_1\) and D\(_2\)-like receptors (Surmeier et al. 1992) suppress the voltage-dependent Na\(^{+}\) currents for generating the action potential itself. The activation of D\(_2\)-like receptors depress the inward Ca\(^{2+}\) currents in isolated striatal neurons (Hernández-López et al. 2000) and in the D\(_2\) or D\(_3\) receptor-expressed cell culture line (Seabrook et al. 1994a,b). Together with the present results, these results suggest that the activation of D\(_1\)- and D\(_2\)-like receptors may thus suppress the firing activity of the medium spiny neurons.

Indirect action of exogenous DA on the medium spiny neurons

Both the DA-induced initial hyperpolarization and slow depolarization were suppressed by bicuculline and atropine, respectively, while they were abolished by TTX. These results indicate that the activations of GABA\(_A\) and muscarinic receptors mediate the initial hyperpolarization and the slow depolarization, respectively, while they also suggest that exogenous DA probably releases endogenous GABA from GABAergic interneurons and/or recurrent axon collaterals from the medium spiny neurons as well as endogenous ACh from cholinergic interneurons. The reversal potentials for the DA-induced initial hyperpolarization and slow depolarization were significantly different (approximately 10 mV) from those for the GABA-induced hyperpolarization and for the ACh-induced depolarization. The negative shift of the reversal potential for the DA-induced initial hyperpolarization may be due to the contamination of anomalous (inward) rectifier K\(^{+}\) currents in GABA-mediated Cl\(^{-}\) currents because the activation of D\(_1\) receptor increases the anomalous K\(^{+}\) current in medium spiny neurons (Pacheco-Cano et al. 1996). This current plays an important role in determining the resting membrane potential and is attributable to Kir2 family channels (Mermelstein et al. 1998). The medium spiny neurons, which expressed D\(_1\) receptor and substance P, have a distinct component of Kir2 channels (Nicola et al. 2000). In the present study, however, the potential change by the activation of anomalous rectifier K\(^{+}\) currents during DA application could not be detected because the K\(^{+}\) equilibrium potential is close to the resting membrane potential. Similarly, it is possible that the positive shift of the reversal potential for the DA-induced slow depolarization is probably due to the contamination of the activation (Freedman and Weight 1988) or suppression (Uchimura and North 1990) of the inward rectifier K\(^{+}\) current via a D\(_2\)-like receptor in the ventral striatal neurons because the equilibrium potential for the inward rectifying current is approximately −80 mV (Uchimura et al. 1989a), which is a more positive value than the resting membrane potential.

Both NMDA- and AMPA-type Glu receptor antagonists suppressed the DA-induced initial hyperpolarization and slow depolarization. The application of exogenous Glu mimicked both the GABA receptor-mediated initial hyperpolarization and the ACh receptor-mediated slow depolarization. The D\(_1\) and D\(_2\)-like receptor agonists enhance Glu-induced firing in rat dorsolateral striatal neurons in vivo (Hu and White 1997) and the D\(_1\)-like receptor activation augments synaptic or ionotropic NMDA receptor-mediated responses in rat or mouse medium spiny neurons in vitro (Cepeda et al. 1993, 1998; Levine et al. 1996a,b). Moreover, presynaptic D\(_2\)-like receptors are present on the Glu-contained nerve terminal in the rat ventral tegmental area where they facilitate local Glu release (Kalivas and Duffy 1995). It is therefore possible that in GABAergic and cholinergic interneurons, the excitatory postsynaptic potentials are augmented by the activation of either D\(_1\)- or D\(_2\)-like receptors on the interneurons and/or an
increase in the Glu release via the presynaptic D1-like receptors on the Glu-containing nerve terminals.

In fact, the cholinergic interneurons contain high levels of D2 and D3 receptors (Bergson et al. 1995) and their mRNA (Yan and Surmeier 1997; Yan et al. 1997). The D1-like receptor agonists enhance the ACh release in the rat striatum (Consolo et al. 1992; Damsma et al. 1990). In neostriatal slices, the cholinergic interneurons show a less negative resting membrane potential (−57 mV) (Kawaguchi 1992, 1993) than that of the medium spiny neurons, and the interneuron is depolarized by the activation of D1-like receptors (Aosaki et al. 1998). As a result, it is most likely that the DA-induced slow depolarization is due to the ACh release induced by the activation of D1- and D2-like receptors in the cholinergic interneurons. On the other hand, a small number of parvalbumin-positive GABAergic interneurons (low-threshold spike cell which contains somatostatin) in vitro also shows a less negative resting membrane potential (−56 mV) (Kawaguchi 1992, 1993). It is likely that the DA-induced initial hyperpolarization is due to the GABA release induced by the activation of D1- and D2-like receptors in the GABAergic interneurons.

In summary, the present study clearly demonstrated that application of exogenous DA for 1 min induced an initial hyperpolarization with a duration of approximately 1 min and a subsequent slow depolarization or slow hyperpolarization with a duration of more than 20 min in the neostriatal medium spiny neurons. These results suggest that the initial hyperpolarization is produced by the activation of the D1- and D2-like receptors in the GABAergic interneurons, whereas the slow depolarization is mainly induced by the activation of D1-like receptors in the cholinergic interneurons and also suggests that the slow hyperpolarization is due to the suppression of intrinsic nonactivating Na\(^+\)-Ca\(^{2+}\) conductances mainly via D2-like receptors in the medium spiny neurons. Taken together, the preceding findings indicate that the DA action for short periods (within 1 min) is a transient inhibition mainly via the GABAergic interneurons, while DA action for long periods (more than 20 min) is a tonic excitation and/or inhibition induced by the activation of mainly D1-like receptors in cholinergic interneurons and by the activation of mainly D2-like receptors in the medium spiny neurons, respectively.

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