Activation of D2-Like Dopamine Receptors Reduces Synaptic Inputs to Striatal Cholinergic Interneurons

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Dopamine (DA) plays a crucial role in the modulation of striatal function. Striatal cholinergic interneurons represent an important synaptic target of dopaminergic fibers arising from the substantia nigra and cortical glutamatergic inputs. By means of an electrophysiological approach from corticostriatal slices, we isolated three distinct synaptic inputs to cholinergic interneurons: glutamate-mediated EPSPs, $GABA_A$-mediated potentials, and Acetylcholine (ACh)-mediated IPSPs. We therefore explored whether DA controls the striatal cholinergic activity through the modulation of these synaptic potentials. We found that SKF38393, a D1-like receptor agonist, induced a membrane depolarization (also see Aosaki et al., 1998) but had no effects on glutamatergic, GABAergic, and cholinergic synaptic potentials. Conversely, D2-like DA receptor activation by quinpirole inhibited both $GABA_A$ and cholinergic synaptic potentials. These effects of quinpirole were mimicked by ω-conotoxin GVIA, blocker of N-type calcium channels. The lack of effect both on the intrinsic membrane properties and on exogenously applied GABA and ACh by quinpirole supports a presynaptic site of action for the D2-like receptor-mediated inhibition. Moreover, the quinpirole-induced decrease in amplitude was accompanied by an increase in paired pulse facilitation ratio (EPSP2/EPSP1), an index of a decrease in transmitter release. Our findings demonstrate that DA modulates the excitability of cholinergic interneurons through either an excitatory D1-like-mediated postsynaptic mechanism or a presynaptic inhibition of the GABAergic and cholinergic inhibitory synaptic potentials.

Key words: dopamine; striatum; electrophysiology; GABA; acetylcholine; EPSP; IPSP

Cholinergic interneurons are relatively large (20–50 μm) aspiny neurons accounting for <5% of the total neuronal population of the striatum and express both D1- and D2-like dopamine (DA) receptors (Le Moine et al., 1990, 1991). Recently, Yan and coworkers (1997) have shown that the large majority (90%) of these interneurons express D5 receptor mRNA rather than D1; likewise, D3 and D4 mRNAs were undetectable, whereas all the interneurons expressed D2 mRNA. Two main physiological effects of DA on striatal cholinergic interneurons are attributed to the stimulation of postsynaptic D1-like DA receptors: depolarization and inward current with an increase, a decrease, or no change in membrane conductance (Aosaki et al., 1998); and enhancement of $GABA_A$-mediated currents (Yan and Surmeier, 1997). Conversely, D2-like DA receptor stimulation has been found to reduce N-type calcium currents (Yan et al., 1997). These experimental data suggest a complex modulation of DA on cholinergic tone in the striatum, via inhibitory and excitatory actions. Striatal cholinergic interneuron activity is also controlled by synaptic excitatory and inhibitory inputs. Summation of only two or three excitatory synaptic potentials is sufficient to trigger an action potential (Wilson et al., 1990; Bennett and Wilson, 1998). During *in vivo* recordings, cholinergic interneurons display a tonic firing discharge activity (Apicella et al., 1991), and thus these cells have been identified as "tonically active neurons." A similar tonic firing discharge activity can also be detected during *in vitro* experiments (Bennett and Wilson, 1998, 1999). It has been shown that the action potential timing of these neurons is sensitive to both excitatory and inhibitory inputs (Bennett and Wilson, 1998). Here, we demonstrate a dopaminergic modulation of these synaptic potentials, which might affect the activity of cholinergic cells, thereby modifying the tone of striatal ACh.

MATERIALS AND METHODS

Preparation and maintenance of the corticostriatal slices. Male Wistar rats (20–30 postnatal d) were used for the experiments. Preparation and maintenance of the slices have been described in detail previously (Calabresi et al., 1997, 1998; Pisani et al., 1999). Briefly, animals were killed under ether anesthesia by cervical dislocation, the brain was removed, and corticostriatal coronal slices (180–200 μm thick) were cut from tissue blocks with the use of a vibratome in an ice-cold (0°C) Krebs' solution (see composition below). A single slice was transferred into a recording chamber mounted on the stage of an upright microscope (Axioskop FS; Zeiss, Thornwood, NY), equipped with a 60×, 0.90 numerical aperture water immersion objective (LUMPlan FI; Olympus Optical, Tokyo, Japan), and fully submerged in a continuously flowing Krebs' solution

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(33°C, 3 ml/min) gassed with 95% O_2/5% CO_2. The composition of the solution was (in mM): 126 NaCl, 2.5 KCl, 1.3 $MgCl_2$, 1.2 NaH_2PO_4, 2.4 $CaCl_2$, 10 glucose, and 18 $NaHCO_3$.

Electrophysiological recordings. Sharp microelectrodes were filled with 2 M KCl (40–60 MΩ). In few cases, potassium acetate (2 M) was used as intraelectrode solution (60–90 MΩ). An Axoclamp 2A amplifier was used for current-clamp recordings. Traces were displayed on an oscilloscope and stored in a digital system. For synaptic stimulation, bipolar electrodes were located either in the cortex or within the striatum to activate corticostriatal fibers or intrastriatal nerve terminals, respectively. Synaptic potentials were measured by averaging responses to four or eight stimuli. Cholinergic neurons were impaled under visual guidance, according to their characteristic shape and size, up to 50–70 μm beneath the surface of the slice. In most of the experiments biocytin, at a concentration of 2–4%, was added to the intraelectrode solution to stain the neurons (Calabresi et al., 1998).

Data analysis and drug application. Values given in the text and in the figures are mean ± SD of changes in the respective cell populations. Student's *t* test (for paired and unpaired observations) was used to compare the means. Drugs were applied by dissolving them to the desired final concentration in the saline and by switching the perfusion from control saline to drug-containing saline after a three-way tap had been turned on. 6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX), (+)-MK 801 maleate (MK-801), and saclofen were from Tocris Cookson (Bristol, UK). Biocytin, L-sulpiride, muscarine, scopolamine, and tetrodotoxin (TTX) were from Sigma (Milan, Italy). GABA, Bicuculline (BMI), methoctramine, picrotoxin, quinpirole, and SKF38393 were from Research Biochemicals (Natick, MA). ω-Conotoxin GVIA was from Alomone Labs (Jerusalem, Israel).

RESULTS

Identification of the recorded cells

Striatal cholinergic interneurons were identified by morphological and electrophysiological criteria (n = 117). In 56 of these 117 cholinergic interneurons the electrophysiological identification was confirmed by a morphological analysis using biocytin. Large aspiny neurons had polygonal or fusiform large somata (25–49 μm), and their dendrites did not show spines. These cells had low membrane potential (−60 ± 3 mV) and high input resistance (155 ± 45 MΩ). Spontaneous firing occurred in 44 cells. In these neurons depolarizing current pulses (100–500 pA) elicited few action potentials followed by a long-lasting afterhyperpolarization (350 ± 130 msec). The amplitude of the action potential was 70.5 ± 3 mV, and the duration of spike at half-amplitude was 0.71 ± 0.05 msec. During hyperpolarizing current pulses (100–400 pA, 2–3 sec), a time-dependent decline in the membrane potential was detected, indicating the presence of a cation current I_h (Fig. 1*A*; n = 80) (Jiang and North, 1991).

Synaptic responses of cholinergic interneurons after cortical stimulation

A single activation of corticostriatal fibers produced EPSPs in most of the recorded cholinergic interneurons (48 of 53). These EPSPs were rather small in amplitude and often triggered an action potential. Thus, in most of the experiments, the cells were hyperpolarized by injecting negative current to hold the membrane potential at approximately −70 mV. Under this experimental condition the EPSP amplitude increased and allowed the characterization of these potentials. Bath application of the NMDA glutamate receptor antagonist MK-801 (30 μM) significantly reduced both the amplitude and the duration of the EPSP, and the subsequent addition of the AMPA glutamate receptor antagonist CNQX (10 μM) further reduced the amplitude of these potentials to 35 ± 13% of the control value (Fig. 1*B*; n = 53). The complete suppression of the depolarizing potential was obtained by adding 30 μM BMI or 50 μM picrotoxin, two $GABA_A$ receptor antagonists (Fig. 1*B*). To avoid intracellular loading with

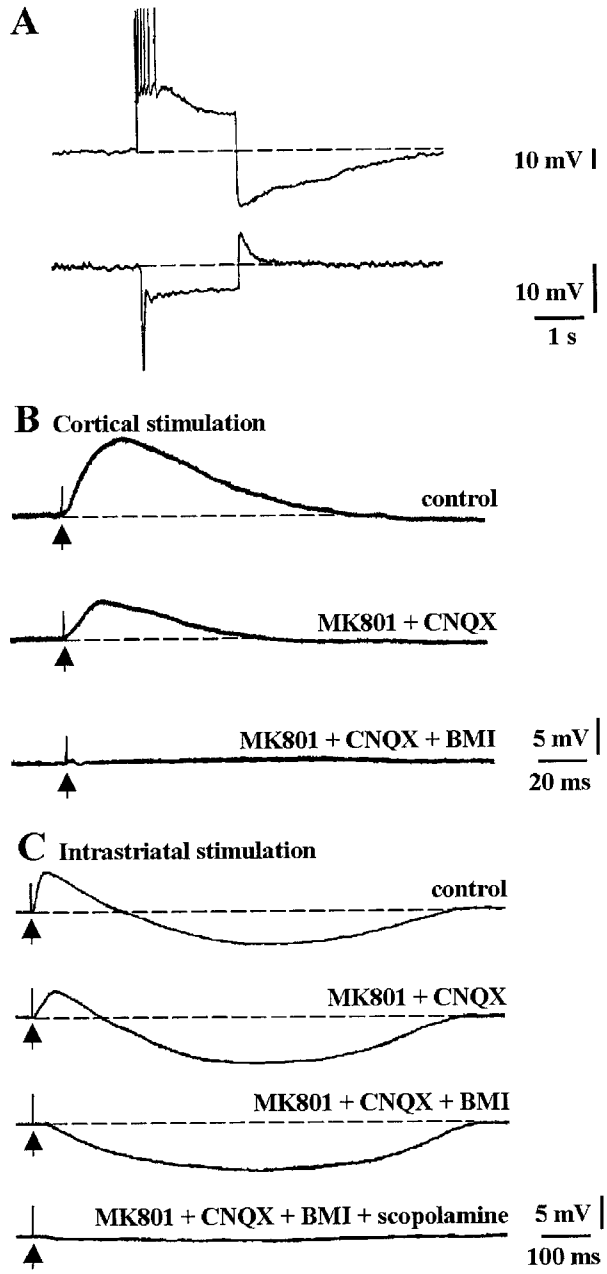

Figure 1. Intrinsic and synaptic properties of striatal cholinergic interneurons. *A, Top,* In a cholinergic interneuron a depolarizing current pulse evoked action potential discharge followed by firing accommodation and a long-lasting afterhyperpolarization (500 pA, 2 sec; note that spikes were truncated). *Bottom,* In the same cell a negative current step (100 pA, 2 sec) induced a voltage response that showed a time-dependent decline. Resting membrane potential (RMP), −60 mV. *B,* In the control condition the stimulation of corticostriatal fibers induced an EPSP. Bath application of the NMDA and AMPA glutamate receptor antagonists MK-801 (30 μM) and CNQX (10 μM) reduced the amplitude of the depolarizing potential. The full suppression of this potential was obtained by adding the $GABA_A$ receptor antagonist BMI (10 μM). RMP, −68 mV. *C,* Stimulation of intrastriatal nerve terminals evoked, in controls, a depolarizing synaptic potential followed by an IPSP. Note that this IPSP was significantly longer than the EPSP. Bath application of the glutamate receptor antagonists CNQX (10 μM) and MK-801 (30 μM) reduced the amplitude of the depolarizing potential, whereas the complete suppression of this potential was obtained by adding the $GABA_A$ receptor antagonist BMI (10 μM). The IPSP was conversely blocked by the muscarinic receptor antagonist scopolamine (1 μM). RMP, −70 mV. Here and in Figures 2–4 the *arrows* indicate when the synaptic stimulus was delivered.

the intraelectrode Cl^- and the substantial change of the reversal potential of this ion, in some experiments ($n = 6$) the electrodes were filled with potassium acetate. In these cases the $GABA_A$ component of the synaptic potentials was either absent or detected as a hyperpolarizing event (1–3 mV).

Synaptic responses of cholinergic interneurons after intrastriatal stimulation

Intrastriatal electrical stimulation also evoked EPSPs resulting from the activation of both glutamate receptors and $GABA_A$ receptors (49 of 51 cells). These potentials were more evident when the cell was hyperpolarized to approximately −72 mV and, compared with those evoked by cortical stimulation, displayed a more pronounced $GABA_A$-mediated component. The blockade of both NMDA- and AMPA-mediated glutamatergic potentials by 30 μM MK-801 and 10 μM CNQX, in fact, suppressed only 30 ± 8% of the amplitude of the EPSP recorded in control solution (Fig. 1*C*; $n = 49$). The full suppression of this potential was obtained by adding 30 μM BMI (Fig. 1*C*). In 22 of these 49 tested neurons, the EPSP was followed by an IPSP. This potential ranged from 3 to 12 mV in amplitude and from 450 to 900 msec in duration depending on the membrane potential of the cell and on the stimulus intensity. As previously described (Calabresi et al., 1998), this IPSP resulted from an increase in membrane potassium conductance and was completely blocked by the muscarinic receptor antagonists scopolamine (1 μM; Fig. 1*C*) and methoctramine (200 nM), an M2-like muscarinic receptor-preferring antagonist (data not shown).

D1-like DA receptor activation on membrane and synaptic potentials of striatal cholinergic interneurons

Bath application of the D1-like DA receptor agonist SKF38393 (1–10 μM) produced a small, reversible membrane depolarization (6 ± 2 mV; $n = 4$; $p < 0.01$), whereas the D2-like DA receptor agonist quinpirole (1–10 μM) had negligible effects on the intrinsic membrane properties of the cells ($n = 20$; $p > 0.05$). The depolarizing effect of SKF38393 also persisted in the presence of the sodium channel blocker TTX, suggesting that it is mediated by the activation of somatodendritic D1-like receptors (data not shown; $n = 3$; $p < 0.001$). In agreement with a previous report (Aosaki et al., 1998), demonstrating that D1-like DA receptor activation excites striatal cholinergic interneurons through the modulation of multiple whole-cell membrane conductances, the depolarizing action of D1-like DA receptor stimulation was coupled to a slight or no change in the apparent input resistance of the cells (data not shown).

To study the effects of D1-like DA receptor activation on the glutamate-mediated EPSPs, we stimulated corticostriatal fibers in the presence of 30 μM BMI and 1 μM scopolamine, whereas, to test the D1-like DA receptor agonist SKF38393 on pure $GABA_A$-mediated potentials, we stimulated intrastriatal fibers in the presence of 30 μM MK-801, 10 μM CNQX, and 1 μM scopolamine. SKF38393 (1–10 μM) failed to affect significantly the amplitude of both glutamate- and $GABA_A$-mediated EPSPs recorded after cortical or intrastriatal activation, respectively (1.9 ± 1 and 2 ± 1.3%, respectively; data not shown; $n = 9$; $p > 0.05$). Because of the depolarizing response to SKF38393, measurements were performed after the injection of constant negative current through the recording electrode (up to 200 pA). SKF38393 (1–10 μM; $n = 6$) was also tested on cholinergic IPSPs evoked by intrastriatal stimulation in the presence of 10 μM CNQX, 30 μM MK-801, and 10 μM BMI. In none of these cells was a significant effect detected (1.3 ± 0.5%; data not shown; $p > 0.05$).

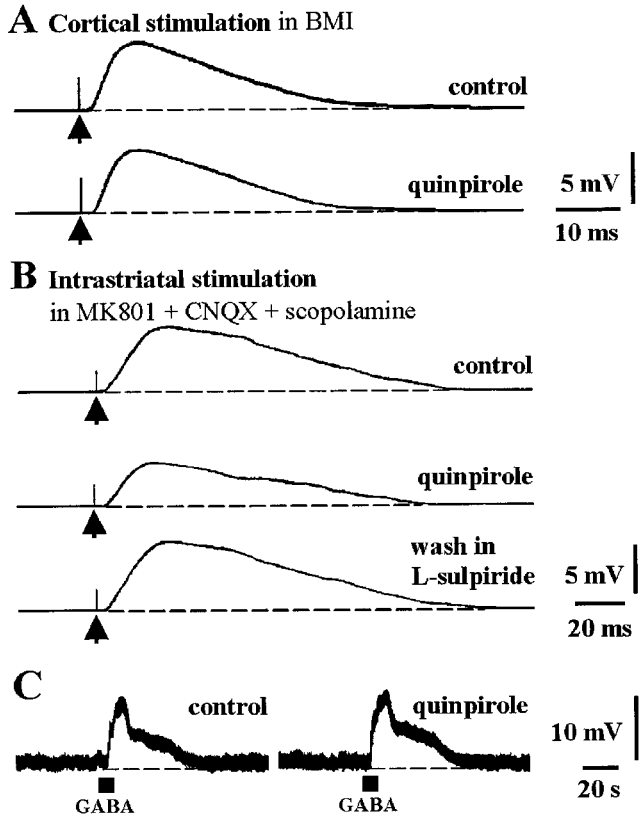

Figure 2. Effects of D2-like DA receptor activation on $GABA_A$-mediated and glutamate-mediated synaptic potentials of striatal cholinergic neurons. *A, Top,* In the presence of 10 μM BMI, stimulation of corticostriatal fibers produced a glutamatergic EPSP in a cholinergic interneuron. *Bottom,* This potential was unaltered by the D2-like DA receptor agonist quinpirole (3 μM, 5 min). RMP, −75 mV. *B, Top,* In the presence of 10 μM CNQX, 30 μM MK-801, and 1 μM scopolamine, intrastriatal stimulation evoked a GABAergic EPSP. *Middle,* This potential was significantly reduced by the D2-like DA receptor agonist quinpirole (3 μM, 5 min) and returned to control value after 10 min washout in the presence of the D2-like DA receptor antagonist L-sulpiride (3 μM; *bottom trace*). RMP, −75 mV. *C, Left,* In the presence of 1 μM TTX plus 500 μM saclofen, exogenous application of GABA (10 mM, 15 sec) produced a membrane depolarization of another cholinergic interneuron. *Right,* Bath application of quinpirole (10 μM, 7 min) failed to affect the amplitude and the duration of the membrane depolarization induced by GABA. RMP, −69 mV.

D2-like DA receptor activation on membrane and synaptic potentials of striatal cholinergic interneurons

In control medium, a dose-dependent inhibition of both cortically and intrastriatally evoked depolarizing potentials was obtained with the D2-like DA receptor agonist quinpirole (1–10 μM; $n = 18$). Interestingly, this action was more pronounced on the EPSPs evoked intrastriatally. Thus, to evaluate the different sensitivity of the two components of the EPSPs to D2-like DA receptor activation, we isolated pharmacologically the glutamate-mediated EPSPs from the GABAergic component. As shown in Figure 2, in most of the recorded neurons, quinpirole (1–10 μM) produced no significant changes of the glutamatergic EPSP ($n = 13$; $p > 0.05$). Conversely, a large and dose-dependent inhibition of the GABAergic EPSP amplitude was observed in all the recorded cells (Fig. 2B; $n = 11$; $p < 0.001$) (1 μM, 11 ± 6%; 3 μM, 43 ± 3%; 10 μM, 55 ± 8%). In most cases the administration of the D2-like DA receptor antagonist L-sulpiride (3 μM), which per se altered

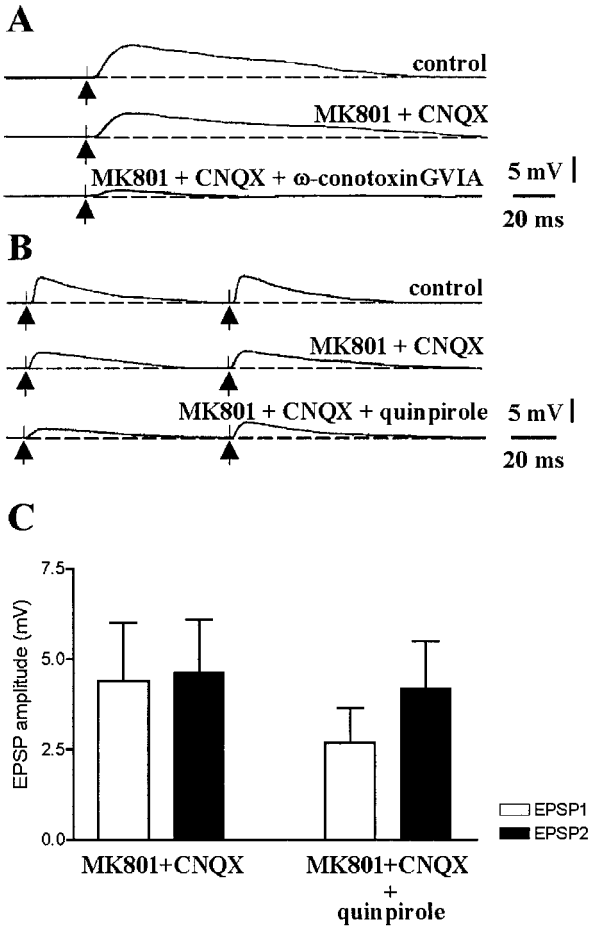

Figure 3. ω-Conotoxin GVIA mimics quinpirole-mediated inhibition, and PPF demonstrates a presynaptic effect. *A,* An intrastriatally evoked EPSP was partially reduced by coadministered 10 μM CNQX and 30 μM MK-801; bath application of 1 μM ω-conotoxin GVIA nearly abolished the GABAergic EPSP. *B,* Synaptic responses to paired stimulation (time interval, 90 msec) in controls, in 10 μM CNQX plus 30 μM MK-801, and in 3 μM quinpirole. Note the increase in the amplitude of the second EPSP in quinpirole compared with controls. *C*, Average amplitude values of pairs of EPSPs before and in the presence of quinpirole.

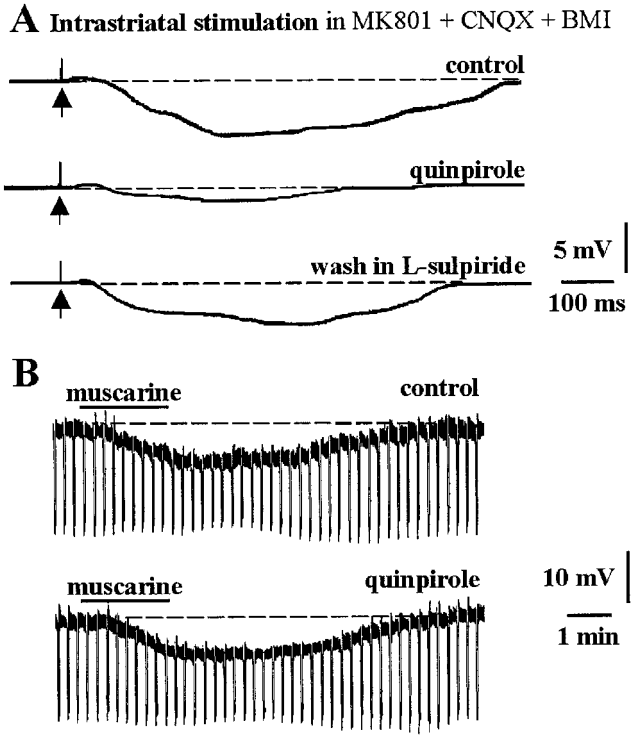

Figure 4. Effects of D2-like DA receptor activation on ACh-mediated synaptic potentials and membrane hyperpolarization induced by muscarine. *A, Top,* In the presence of 10 μM CNQX, 30 μM MK-801, and 10 μM BMI, intrastriatal stimulation evoked an IPSP in a cholinergic interneuron. *Middle,* This potential was primarily reduced by the D2-like DA receptor agonist quinpirole (3 μM, 5 min) and returned to control value after 10 min washout in the presence of the D2-like DA receptor antagonist L-sulpiride (3 μM; *bottom*). RMP, −65 mV. *B, Top,* In the presence of 1 μM TTX, application of 10 μM muscarine (2 min) produced a membrane hyperpolarization of another interneuron. *Bottom,* Bath application of quinpirole (3 μM, 7 min) did not produce any significant effect on the muscarine-induced membrane hyperpolarization of the cell. RMP, −70 mV. Downward deflections are hyperpolarizing electrotonic potentials evoked by current pulses (250 pA, 2 sec). Their decline after the initial peak reflects the expression of a prominent I_h. The muscarine-induced membrane hyperpolarization was coupled to a decreased input resistance (38 ± 6%), which was unaffected by quinpirole.

neither the membrane potential nor the amplitude and duration of synaptic potentials, was required to fully restore, at washout of quinpirole, the control EPSP amplitude (Fig. 2*B*). Furthermore, quinpirole (10 μM) failed to reduce the membrane depolarizations obtained in cholinergic interneurons by application of exogenous GABA (10 mM, 15 sec; Fig. 2*C*), suggesting that the inhibition produced by quinpirole on GABAergic synaptic potentials is mediated by presynaptic D2-like DA receptors.

To investigate the mechanisms underlying the quinpirole-mediated inhibition, we applied ω-conotoxin GVIA, a blocker of N-type high-voltage-activated calcium channels, in the presence of 10 μM CNQX and 30 μM MK-801, on the $GABA_A$-isolated EPSP component. As shown in Figure 3*A*, ω-conotoxin GVIA (1 μM, 5 min) mimicked the effect of quinpirole and nearly abolished the EPSP (80 ± 5%; $n = 6$; $p < 0.001$). This effect was observed independently on the location of the stimulating electrode (cortical vs intrastriatal). Similarly, when tested on the cholinergic IPSP, ω-conotoxin GVIA (1 μM, 5 min) produced complete inhibition of the IPSP amplitude (data not shown; $n = 3$; $p < 0.001$).

Paired pulse facilitation (PPF) is considered an indicator of changes in presynaptic transmitter release, and an increase in the ratio of the second pulse to the first pulse response (EPSP2/EPSP1) indicates a decrease in the release probability (Manabe et al., 1993; Calabresi et al., 1997). Thus, we studied synaptic responses to a pair of stimuli with a time interval of 80–100 msec in controls in the presence of 10 μM CNQX and 30 μM MK-801 and during quinpirole application. Figure 3*B* shows that bath-applied quinpirole (3 μM, $n = 5$) caused an increase in the EPSP2/EPSP1 ratio, further supporting a presynaptic site of action (average ratio values: control, 1.1 ± 0.4; in quinpirole, 1.6 ± 0.3).

Effects of D2-like receptor activation on muscarinic IPSP

We tested then whether quinpirole affected the cholinergic IPSP evoked by intrastriatal stimulation in the presence of 10 μM CNQX, 30 μM MK-801, and 10 μM BMI. As seen for GABAergic potentials, quinpirole (3 μM; $n = 6$) produced a marked inhibition (73 ± 5%; $p < 0.001$) of this IPSP, reversible at the washout in the presence of 3 μM L-sulpiride (Fig. 4*A*). Indeed, quinpirole (1–10

μM) failed to alter the membrane hyperpolarization (4–8 mV; $n = 4$; $p > 0.05$) evoked by bath-applied muscarine (10 μM, 2–3 min; also see Calabresi et al., 1998), indicating that D2-like DA receptor activation inhibits ACh-mediated IPSP through a presynaptic mechanism (Fig. 4*B*).

DISCUSSION

In the present study, we provided evidence for a DAergic modulation of striatal cholinergic interneuron activity through two distinct mechanisms: the D1-like DA receptor-mediated membrane depolarization of these cells and the D2-like-dependent presynaptic inhibition of synaptic potentials, principally the inhibitory GABAergic and muscarinic components. Among the different nerve terminals contacting striatal interneurons, glutamatergic, GABAergic, and cholinergic fibers mediate synaptic potentials in cholinergic interneurons (Kawaguchi, 1992; Bennett and Wilson, 1998; Calabresi et al., 1998). Glutamatergic fibers mainly originate from the cerebral cortex and the thalamus (Lapper and Bolam, 1992; Matsumoto et al., 1996), whereas the sources of both GABAergic and cholinergic inputs are intrinsic (Bolam et al., 1986). Recurrent axon collaterals of GABAergic projection cells or other striatal interneurons that use GABA as neurotransmitter provide the GABA-mediated synaptic potentials in cholinergic cells (Bolam et al., 1986; Yan and Surmeier, 1997). Recently it has been shown that cholinergic interneurons also reduce their own excitability through a peculiar mechanism, a slow muscarinic IPSP (Calabresi et al., 1998).

DA is a crucial regulator of striatal function. Loss of nigrostriatal DAergic projection causes severe motor abnormalities in Parkinson's disease patients and in animal models of parkinsonism. Despite this clinical and experimental evidence, the cellular mechanisms by which DA affects striatal neuron activity are still for the most part unknown.

It is now accepted that the activation of different DA receptor subtypes elicits distinct effects on cholinergic interneurons (Stoof et al., 1992; Yan and Surmeier, 1997; Yan et al., 1997; Aosaki et al., 1998) that represent the main source of ACh in the striatum (Bolam et al., 1984). Indeed, the D2 receptor-mediated reduction of N-type calcium currents described by Yan and coworkers (1997) may well account for our observation of a D2-like-mediated inhibition of both the GABAergic component of the EPSP and the muscarinic IPSPs. These quinpirole-mediated effects were in fact mimicked by ω-conotoxin GVIA, indicating an involvement of N-type calcium channels in the inhibitory action of quinpirole. Interestingly, in the large majority of the cells the negative modulation by D2-like receptors on the glutamatergic component of the EPSP was absent, whereas a small inhibitory effect was observed only in a subset of cells. These seemingly conflicting results might be explained considering that cholinergic cells receive glutamatergic inputs from different sources (Lapper and Bolam, 1992; Matsumoto et al., 1996); hence, the nerve terminals impinging on these cells might exhibit heterogeneous expression and sensitivity of presynaptic D2-like receptors.

The observation that D1-like receptor activation causes a membrane depolarization is in agreement with the observation by Aosaki and coworkers (1998), who reported an excitatory effect by D1-like receptor agonists. Recently, an enhancement of $GABA_A$ currents by D1-like receptor activation has been reported (Yan and Surmeier, 1997), whereas we failed to detect a modulation by SKF38393 on the isolated $GABA_A$-mediated component of the EPSP. This apparent discrepancy between the present results and the work by Yan and Surmeier (1997) may reside in the different experimental tissue preparation used. One possibility is, in fact, that in our experimental condition, i.e., slice versus dissociated cell preparation, synaptically evoked $GABA_A$ potentials originate from a set of conductances located on dendritic branches, whose sensitivity to DA may differ from that of those originating at the somatic level.

Our evidence of an increase in the EPSP2/EPSP1 ratio in PPF experiments by quinpirole supports a D2-like-dependent presynaptic inhibition of both GABAergic and cholinergic inhibitory potentials, which might ultimately lead to a DA-induced disinhibition of cholinergic interneuron activity. The latter effect together with the D1-like-mediated direct membrane depolarization would suggest a final excitatory DAergic drive on cholinergic cells. This view is partially in contrast with previous work demonstrating an inhibitory effect of D2 receptors on striatal cholinergic function (Stoof et al., 1992; DeBoer et al., 1996). It is reasonable, in fact, to assume that the D2-like-dependent inhibition of calcium currents through N-type channels would attenuate the dendritic invasion of initial segment spikes (Spruston et al., 1995) and the summation of excitatory synaptic inputs from thalamic and cortical afferents, thereby reducing the excitability of these cells. Apparently, the complexity of striatal circuitry accounts for the difficulty of clarifying the role of DA in striatal functioning; additional work is required to address this issue.

REFERENCES

Aosaki T, Kiuchi K, Kawaguchi Y (1998) Dopamine D1-like receptor activation excites rat striatal large aspiny neurons in vitro. J Neurosci 18:5180–5190.

Apicella P, Scarnati E, Schultz W (1991) Tonically discharging neurons of monkey striatum respond to preparatory and rewarding stimuli. Exp Brain Res 84:672–675.

Bennett BD, Wilson CJ (1998) Synaptic regulation of action potential timing in neostriatal cholinergic interneurons. J Neurosci 18:8539–8549.

Bennett BD, Wilson CJ (1999) Spontaneous activity of neostriatal cholinergic interneurons in vitro. J Neurosci 19:5586–5596.

Bolam JP, Wainer BH, Smith AD (1984) Characterization of cholinergic neurons in the rat striatum. A combination of choline acetyltransferase immunocytochemistry, Golgi impregnation and electron microscopy. Neuroscience 12:711–718.

Bolam JP, Ingham CA, Izzo PN, Levey AI, Rye DB, Smith AD, Wainer BH (1986) Substance P-containing terminals in synaptic contact with cholinergic neurons in the neostriatum and basal forebrain: a double immunocytochemical study in the rat. Brain Res 397:279–289.

Calabresi P, Ascone Magarinos C, Centonze D, Pisani A, Sancesario G, D'Angelo V, Bernardi G (1997) Opposite membrane potential changes induced by glucose deprivation in striatal spiny neurons and in large aspiny interneurons. J Neurosci 17:1940–1949.

Calabresi P, Centonze D, Pisani A, Sancesario G, North RA, Bernardi G (1998) Muscarinic IPSPs in rat striatal cholinergic interneurons. J Physiol (Lond) 510:421–427.

DeBoer P, Heeringa MJ, Abercrombie ED (1996) Spontaneous release of acetylcholine in striatum is preferentially regulated by inhibitory dopamine D2 receptors. Eur J Pharmacol 317:257–262.

Jiang ZG, North RA (1991) Membrane properties and synaptic responses of rat striatal neurones in vitro. J Physiol (Lond) 443:533–553.

Kawaguchi Y (1992) Large aspiny cells in the matrix of the rat neostriatum in vitro: physiological identification, relation to the compartments and excitatory postsynaptic currents. J Neurophysiol 67:1669–1682.

Lapper SR, Bolam JP (1992) Input from the frontal cortex and the parafascicular nucleus to cholinergic interneurons in the dorsal striatum of the rat. Neuroscience 51:533–545.

Le Moine C, Tison F, Bloch B (1990) D2 dopamine receptor gene expression by cholinergic neurons in the rat striatum. Neurosci Lett 117:248–252.

Le Moine C, Normand E, Bloch B (1991) Phenotypical characterization of the rat striatal neurons expressing the D1 dopamine receptor gene. Proc Natl Acad Sci USA 88:4205–4209.

Manabe T, Wyllie DJI, Nicoll RA (1993) Modulation of synaptic trans-

mission and long-term potentiation: effects on paired-pulse facilitation and EPSC variance in the CA1 region of the hippocampus. J Neurophysiol 70:1451–1459.

Matsumoto N, Minamimoto T, Kimura M (1996) Role of thalamostriatal projection in acquisition of striate neuron activity through behavioral learning in the monkey. Neurosci Res Suppl 20:2318.

Pisani A, Calabresi P, Centonze D, Marfia GA, Bernardi G (1999) Electrophysiological recordings and calcium measurements in striatal large aspiny interneurons in response to combined O_2/glucose deprivation. J Neurophysiol 81:2508–2516.

Spruston N, Schiller Y, Stuart G, Sakmann B (1995) Activity-dependent action potential invasion and calcium influx into hippocampal CA1 dendrites. Science 268:297–300.

Stoof JC, Drukarch B, De Boer P, Westerink BHC, Groenewegen HJ (1992) Regulation of the activity of striatal cholinergic neurons by dopamine. Neuroscience 47:755–770.

Wilson CJ, Chang HT, Kitai ST (1990) Firing patterns and synaptic potentials of identified giant aspiny interneurons in the rat neostriatum. J Neurosci 10:508–519.

Yan Z, Surmeier DJ (1997) D5 dopamine receptors enhance Zn^{2+}-sensitive GABAA currents in striatal cholinergic interneurons through a PKA/PP1 cascade. Neuron 19:1115–1126.

Yan Z, Song W-J, Surmeier DJ (1997) D2 dopamine receptors reduce N-type Ca^{2+} currents in rat neostriatal cholinergic interneurons through a membrane-delimited, protein-kinase-C-insensitive pathway. J Neurophysiol 77:1003–1015.