Dopamine Excites Fast-Spiking Interneurons in the Striatum

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INTRODUCTION

The striatum is the main synaptic target of mesencephalic dopaminergic neurons (Joel and Weiner 2000). Decrease in striatal dopamine results in Parkinson’s disease severe motor disorders (Obeso et al. 2000). The striatum is also a major site of action for psychostimulants such as amphetamine and cocaine, which increase extracellular dopamine concentration, (Koob 2000). Striatal neurons bear dopamine receptors, which are grouped as D1-like and D2-like (Sealfon and Olanow 2000). In vitro experiments in rodents revealed that the action of dopamine on striatal projection neurons is a complex one, because it does not change resting membrane potential but affects several voltage-dependent conductances (Calabresi et al. 2000a; Nicola et al. 2000) and modulates both inhibitory and excitatory synaptic inputs and long-term synaptic plasticity (Calabresi et al. 2000b; Delgado et al. 2000; Levine and Cepeda 1998; Tang et al. 2001). Another potential target for dopaminergic action are striatal interneurons. The ability of these cells, which include cholinergic and GABAergic neurons, to control striatal operation has recently emerged. Selective ablation of cholinergic interneurons results in severe behavioral deficits in vivo (Kaneko et al. 2000). Dopamine strongly excites cholinergic interneurons through D1-like receptors (Aosaki et al. 1998) and depresses GABAergic and cholinergic input to these neurons through presynaptic D2-like receptors (Momiyama and Koga 2001; Pisani et al. 2000). GABAergic inhibition strongly limits projection neuron activity in vivo (Nisenbaum and Berger 1992) and appears to arise mainly from interneurons (Koos and Tepper 1999). Dopaminergic modulation of GABAergic interneurons may therefore provide an important striatal control mechanism. The present study investigated the effects of dopamine on a well-identified class of striatal GABAergic interneurons, the fast-spiking (FS) interneurons (Kawaguchi et al. 1995).

METHODS

Male Wistar rats (25–40 postnatal day) were used as previously described (Calabresi et al. 2000b). Briefly, animals were killed under ether anesthesia, the brain was quickly removed, and corticostriatal coronal slices (200- to 300-μm thick) were cut and maintained at 34°C in oxygenated artificial cerebrospinal solution (ACSF; composition, in mM: 126 NaCl, 2.5 KCl, 1.3 MgCl₂, 1.2 NaH₂PO₄, 2.4 CaCl₂, 10 glucose, and 18 NaHCO₃). For recordings, slices were transferred to a submerged chamber and continuously superfused (2–3 ml/min) at 34°C; for whole cell recordings, neurons were visualized with differential interference contrast microscopy. FS interneurons were medium-sized cells and could not be visually distinguished from projection neurons. Patch pipettes (2–5 MΩ) were filled with intracellular solution containing (in mM) 125 KCl, 34 K-acetate, and 40 MES with 0.2 M KCl added to pH 7.3 with KOH. For sharp electrode recordings, pipettes (30–60 MΩ) were filled with 2 M KCl. Voltage-clamp recordings were performed in whole cell patch-clamp configuration with an Axopatch 1D amplifier (Axon Instruments). Whole cell access resistance was 5–30 MΩ before electronic compensation (60–80%). Current-clamp recordings were performed in bridge mode with an Axoclamp-2B. Drugs were bath-applied at the following concentrations: (+)-MK 801 maleate (MK-801) and dopamine, 30 μM; cocaine, 20 μM; bicuculline (BMI), 3 μM; quinpirole, SCH 23390, SKF38393, t-sulpiride, and 6-cyano-7-nitroquinoxaline-2-3-dione (CNOX), 10 μM; tetrodotoxin (TTX), 1 μM; GABA, 100 μM. To prevent oxidation, dopamine was dissolved into continuously gassed ACSF immediately before bath application. To study the effects of drugs on membrane potential and input...
resistance, drugs were applied for 4–10 min. Statistical significance of membrane potential changes was assessed by comparison of the maximal membrane potential change induced by a certain treatment with the maximal variation observed during a period of 10 min without drugs in the same cells. Membrane input resistance was monitored with negative current steps (100–300 pA, 500 ms) delivered at 0.1 Hz; during depolarizing responses, measurements were made when the cell was briefly repolarized by negative current injection (100–300 pA) to pretreatment level. For each cell, 3–10 steps delivered in the presence of a given treatment were compared with 10 steps measured in control solution to detect statistical differences.

Electrical stimuli (0.1 ms, 5–10 V) were delivered with a bipolar tungsten electrode placed in the white matter between the cortex and the striatum to evoke excitatory postsynaptic currents (EPSCs) or intrastriatally to evoke inhibitory postsynaptic currents (IPSCs) under voltage-clamp conditions. EPSCs were evoked at ~80 mV in the presence of the GABA_A receptor antagonist bicuculline and were mediated by ionotropic glutamate receptors because they were blocked by co-application of the non-N-methyl-D-aspartate (NMDA) glutamate receptor antagonist CNQX and the NMDA glutamate receptor antagonist MK-801. IPSCs were recorded at 0 mV in the presence of CNQX plus MK-801 and were mediated by GABA_A receptors because they were blocked by bicuculline. Stimuli were continuously delivered at 0.1 Hz. The effects of drugs on evoked EPSCs and IPSCs were measured 8 min after start of bath application. Washout data were collected 15 min after the end of drug application. In the experiments in which dopamine antagonists were co-applied with dopamine or cocaine, the antagonist was applied 8 min after the start of dopamine or cocaine application, and relative measurements were made 15 min after the start of the co-application. In each neuron, 10 consecutive EPSC/IPSCs were measured for each experimental condition. For each cell, data were normalized by dividing the peak amplitude of each response by the average response recorded just before drug application. Normalized data from different cells for each experimental condition (including control) were then pooled to perform statistical. All values are expressed as means ± SD and statistical comparisons were performed by Student’s t-test.

RESULTS

FS interneurons (n = 25) were readily identified based on their distinctive electrophysiological properties, which markedly differed from those of projection neurons (Fig. 1) and of other striatal interneurons (Kawaguchi et al. 1995; Koos and Tepper 1999). FS interneurons had resting membrane potential (RMP) close to −80 mV, were silent at rest, and displayed high maximal firing rate (≤200 Hz) with little adaptation. Spikes were short and followed by large afterhyperpolarizations, and intermittent burst firing was observed in response to moderate positive current steps (Fig. 1B). These properties were similar in FS interneurons recorded with patch (n = 12) or sharp microelectrodes (n = 13). With patch electrodes, RMP was −81 ± 6 mV and input resistance 101 ± 35 MΩ; with sharp electrodes, RMP was −77 ± 7 mV and input resistance 43 ± 11 MΩ.

Effects of dopamine and cocaine on FS interneuron membrane potential

The effects of dopamine on FS interneuron membrane potential were studied under current-clamp conditions with sharp microelectrodes to minimize disturbance to the intracellular environment. In all cases (n = 6), dopamine application (4–8 min) elicited a reversible membrane depolarization (peak amplitude 7.1 ± 2.5 mV, Fig. 2A). This depolarization was accompanied by a significant (P < 0.05) increase in input resistance (29 ± 15%). When dopamine was re-applied >20 min after washout, it elicited effects similar to those of the first application. In 4/6 cells, the early phase of the dopamine-induced depolarization was accompanied by the generation of action potentials (Fig. 2A). This phenomenon was not simply attributable to the slow dopamine-induced depolarization because such depolarization per se was below spike threshold as suggested by the observation that action potentials were not generated during the late phase of the dopamine effects even though the depolarization was similar or even larger (as in the example of Fig. 2A). Thus spontaneous synaptic activity superimposed on dopamine-induced depolarization (and amplified by increased input resistance) appeared to be mainly responsible for spike generation.

Dopamine elicited a depolarization (6.8 ± 2.3 mV) and a significant increase in input resistance (24 ± 9%; P < 0.05) also when applied in the presence of the sodium channel blocker TTX (n = 3), showing that these effects were caused by a direct action on FS interneurons.

Bath application of cocaine also caused a membrane depolarization (5.8 ± 1.5 mV) in 5/5 FS interneurons, accompanied by a significant (P < 0.05) increase in input resistance (19 ± 11%) as illustrated in Fig. 2B. These data suggest that an increase of endogenous extracellular dopamine elicits similar effects to bath-applied dopamine, although increases in other
biogenic amines (Blakely and Bauman 2000) might also have played a role. To establish whether dopamine acted through D1- and/or D2-like receptors, we used selective agonists. The D2-like receptor agonist quinpirole did not elicit significant changes in membrane potential or input resistance \((n=11005)\); conversely, the D1-like receptor agonist SKF38393 produced effects similar to those of dopamine, depolarizing 4/4 FS interneurons \((5.9/110062.3 \text{ mV})\) and significantly \((P<0.05)\) increasing their input resistance (by 23/1100613%). To further confirm the involvement of D1-like receptors in dopamine-induced depolarization, we bath applied the D1-like receptor antagonist SCH23390 \((n=11005)\) or the D2-like receptor antagonist L-sulpiride \((n=11005)\). Neither of these agents significantly affected per se FS interneuron membrane potential or input resistance (Fig. 2, C and D). In the presence of SCH23390, application of dopamine or cocaine failed to produce significant effects on membrane potential or input resistance. In the presence of L-sulpiride, dopamine induced a depolarization similar to that observed in control \((6.6 \pm 2.1 \text{ mV})\) and significantly \((P<0.05)\) increased input resistance (by \(18 \pm 12\%\)). A summary of the effects of all drugs applied on membrane potential and input resistance (obtained by pooling all cells tested in each pharmacological condition) is shown in Fig. 2, C and D.

**Effects of dopamine on evoked synaptic currents**

Evoked synaptic currents were studied with patch pipettes in voltage-clamp (see Methods). Under these conditions, at \(-80 \text{ mV}\), dopamine and SKF38393 elicited modest and inconsistent inward currents, presumably due to intracellular dialysis interfering with dopamine-induced metabolic cascades. Dopamine did not cause any detectable change in evoked glutamatergic currents \((n=5)\) as shown in Fig. 3A.

![Figure 3](image-url) **FIG. 3. Effects of dopamine on evoked synaptic currents.** A: inward glutamatergic currents were not affected by 30/9262M dopamine (each trace in A–C is the average of 5 consecutive sweeps). B: outward GABAergic currents were depressed by 30/9262M dopamine but recovered to control level when 10/9262M L-sulpiride was co-applied. C: 20\text{ M} cocaine reversibly depressed GABAergic currents. D: time course of evoked inhibitory postsynaptic currents (IPSCs) amplitude during 30\text{ M} dopamine (DA) application and subsequent 10\text{ M} l-sulpiride co-application for an individual experiment (left) and for the average of 4 experiments (right). For each time point, data from each cell are normalized to the average of control IPSC amplitude. Error bars in D and E represent SD. E: quantification of the effects of various treatment on GABAergic current amplitude. Double asterisk, statistical significance \((P<0.001)\). sulp, L-sulpiride; coc, cocaine.
Similarly, quinpirole \((n = 3)\) or SKF38393 \((n = 3)\) failed to affect glutamatergic responses. Conversely, GABAAergic currents were significantly \((P < 0.001; \text{see methods for details of data collection})\) reduced in amplitude \((61 \pm 6\%\) of the average amplitude observed in control ACSF\) by dopamine \((n = 7)\); these effects were fully reversed after 15-min washout, and a new application of dopamine delivered 20–25 min after the end of the previous application elicited similar effects. Dopamine effects were also reversed by subsequent co-application of L-sulpiride \((n = 4; \text{Fig. 3B})\) but not of SCH23390 \((n = 3)\). The time course of the effects of dopamine and of the subsequent co-application of L-sulpiride on evoked IPSCs peak amplitude are illustrated in Fig. 3D for an individual experiment \((\text{left})\) and for the average of 4 experiments \((\text{right})\). GABAAergic currents were also significantly \((P < 0.001)\) reduced \((57 \pm 6\%\) of control) by quinpirole \((n = 4)\) but not by SKF38393 \((n = 4)\), confirming that D2-like but not D1-like receptors were involved in this phenomenon \((\text{Fig. 3E})\). Cocaine also induced a significant \((P < 0.001)\) and reversible decrease \((69 \pm 7\%\) of control) in GABAAergic current amplitude \((n = 4)\). Cocaine effects on GABAAergic currents were blocked by L-sulpiride \((n = 3)\). The effects of the drugs tested are summarized in Fig. 3E. Bath application of GABA \((20 \text{ s})\) elicited an outward current \((352 \pm 150 \text{ pA peak amplitude})\) in FS interneurons voltage-clamped at 0 mV; this outward current was not significantly affected by dopamine \((n = 3)\), suggesting that the observed effects were due to a presynaptic action.

**DISCUSSION**

The present study revealed that dopamine directly depolarizes FS interneurons in the striatum via D1-like receptors. Furthermore, it decreases the inhibitory synaptic input to these cells via presynaptic D2-like receptors. Similar effects are also elicited by cocaine, suggesting that it can increase extracellular endogenous dopamine \((\text{Blakely and Bauman 2000})\) to levels sufficient to activate both D1 receptors on FS interneurons and D2 receptors on GABAAergic presynaptic terminals impinging on these cells. However, elevated extracellular concentration of other biogenic amines \((\text{Blakely and Bauman 2000})\) may also have contributed to the observed effects of cocaine. D1-like receptor-mediated depolarization was often accompanied by an increase in membrane resistance, suggesting that one of the ionic mechanisms involved might be a decrease in a potassium conductance. Action potentials were often observed during the early phase of dopamine application and appeared to be mainly due to ongoing spontaneous EPSP (amplified by increased input resistance) superimposed on dopamine-induced depolarization. However, activation of gap junctions between FS interneurons \((\text{Koos and Tepper 1999})\) could also have contributed to spike triggering. Further investigation will be required to clarify this issue.

The origin of the GABAAergic input to FS interneurons remains to be clarified because dual recordings suggested that it may not come from projection neurons or from FS interneurons \((\text{Koos and Tepper 1999})\), and pallidal afferents may be involved \((\text{Bevan et al. 1998})\). D2-like receptor-mediated depression of GABAAergic IPSCs on FS interneurons is similar to that observed in projection neurons \((\text{Delgado et al. 2000})\) and cholinergic interneurons \((\text{Momiyama and Koga 2001; Pisani et al. 2000})\).

FS interneurons are well identified as parvalbumin-containing GABAAergic cells \((\text{Kawaguchi et al. 1995})\), and powerfully inhibit projection neurons, which conversely do not appear to inhibit each other significantly \((\text{Jaeger et al. 1994})\). Thus, FS interneurons \((\text{and low-threshold spike interneurons})\) \((\text{Koos and Tepper 1999})\) are probably the main neurons responsible for striatal GABAAergic inhibition; this strongly limits striatal output in vivo \((\text{Nisenbaum and Berger 1992})\). It is therefore evident that, by exciting FS interneurons and by reducing their synaptic inhibition, dopaminergic afferents can exert a major inhibitory influence on the striatum. In particular, dopamine may critically regulate feed-forward inhibition, which is a primary feature of cortico-striatal communication \((\text{Koos and Tepper 1999})\). The present results also provide a cellular explanation for in vivo evidence that striatal GABA release is increased by D1-like receptor agonists and decreased by D2-like receptor antagonists \((\text{Harsing and Zigmund 1997})\).

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