Neuron, Vol. 42, 939–946, June 24, 2004, Copyright ©2004 by Cell Press

Vesicular Dopamine Release Elicits an Inhibitory Postsynaptic Current in Midbrain Dopamine Neurons

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Summary

Synchronous activation of dopamine neurons, for instance upon presentation of an unexpected rewarding stimulus, results in the release of dopamine from both terminals in projection areas and somatodendritic sites within the ventral midbrain. This report describes an inhibitory postsynaptic current (IPSC) that was elicited by dopamine in slices from mouse midbrain. The IPSC was tetrodotoxin sensitive, calcium dependent, and blocked by a D2 receptor antagonist. Inhibition of monoamine transporters prolonged the IPSC, indicating that the time course of dopamine neurotransmission is tightly regulated by reuptake. Changing the stimulus intensity altered the amplitude but not the time course of the IPSC, whose onset was faster than could be reproduced with iontophoresis. The results indicate a rapid rise in dopamine concentration at the D2 receptors, suggesting that dopamine that is released by a train of action potentials acts in a localized area rather than in a manner consistent with volume transmission.

Introduction

Dopamine cells located in the substantia nigra (SN) and ventral tegmental area (VTA) contribute to reward, learning, and movement and are implicated in drug abuse and a variety of mental disorders. Burst firing of dopamine cells releases dopamine in forebrain terminal regions, including the striatum and limbic cortices (Grillner and Mercuri, 2002; Rice et al., 1997; Tepper et al., 1991), as well as locally in the midbrain through somatodendritic release (Bjorklund and Lindvall, 1975; Geffen et al., 1976). Cell bodies and dendrites of dopamine neurons express D2-like dopamine autoreceptors that decrease excitability (Kim et al., 1995; Lacey et al., 1987). Thus, local release of dopamine likely affects the tonic and phasic firing patterns that these cells demonstrate in vivo (Pucak and Grace, 1994).

Although release of dopamine in the midbrain has been detected, conflicting evidence exists on the mechanisms of that release (Falkenburger et al., 2001; Hoffman and Gerhardt, 1999; Jaffe et al., 1998). Reports on the calcium dependence of dopamine release are mixed, in that both calcium-dependent (Geffen et al., 1976; Cheramy et al., 1981; Chen and Rice, 2001) and calcium-independent (Chen and Rice, 2001; Hoffman and Gerhardt, 1999; Kalivas and Duffy, 1991; Rice et al., 1997) components have been observed. Recent work also suggests that reversal of the dopamine transporter (DAT) may contribute to dopamine release (Falkenburger et al., 2001; Nirenberg et al., 1996b), although other evidence does not support this mechanism (Cragg et al., 1997; Hoffman and Gerhardt, 1999).

Transmitter release from sites without a specialized structural arrangement is thought to mediate volume transmission (Fuxe and Agnati, 1991) or ectopic release (Matsui and Jahr, 2003). Extrasynaptic mechanisms of this kind have been hypothesized for dopaminergic transmission, based largely on the reported extrasynaptic localization of receptors and the small number of dendrodendritic synapses between dopamine cells (Groves and Linder, 1983; Nirenberg et al., 1996a; Wilson et al., 1977; Yung et al., 1995). Somatodendritic release of dopamine is well established (Geffen et al., 1976; Kalivas and Duffy, 1991), and recent work using fast cyclic voltammetry and amperometry have reported both rapid and prolonged kinetics of this release (Cragg et al., 2001; Hoffman and Gerhardt, 1999; Jaffe et al., 1998; Rice, 2000; Rice et al., 1997). However, only through examining the physiological consequences of dopamine release can the kinetics of dopamine transmission in the midbrain be elucidated. Using whole-cell recordings from dopamine cells in slices of ventral midbrain from mouse, we report an inhibitory postsynaptic current (IPSC) that is elicited by somatodendritic release of dopamine. The kinetics of this IPSC and sensitivity of the current to pharmacologic alterations targeting vesicle release suggest that synaptically released dopamine activates D2 receptors that are in close proximity to release sites rather than diffusing in a paracrine fashion consistent with volume transmission.

Results

Identification of a Dopamine Receptor-Mediated IPSC

Whole-cell recordings were made from SN (about 90%) and VTA (10%) dopamine neurons in slices from mouse brain. A bipolar stimulating electrode was placed into the slice just caudal to the cell bodies and was used to evoke transmitter release. Receptor antagonists were used to block $GABA_A$, α-1-adrenoceptors, NMDA, AMPA, and $GABA_B$ receptor-mediated synaptic currents. In the presence of these blockers, electrical stimulation directly activated dopamine cells and produced a reproducible slow IPSC that was eliminated by the D2 receptor antagonist sulpiride (50–150 nM; Figures 1A and 1B). This IPSC was detected in approximately 95% of the cells tested and varied between 20 and 350 pA in amplitude, depending on the proximity of the stimulating electrodes and the number of stimuli applied. An IPSC could be evoked by a single stimulation, but for most experiments a train of stimuli (five stimuli at 40 Hz) was applied

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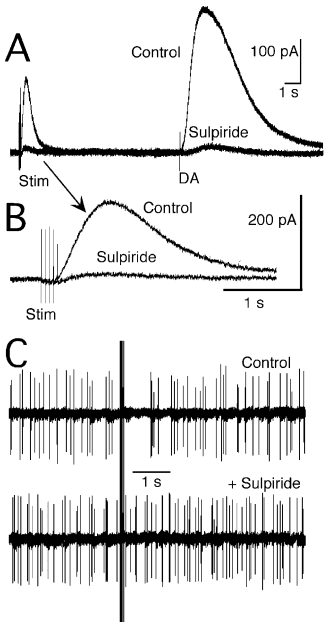

Figure 1. Electrical Stimulation Evokes a Sulpiride-Sensitive IPSC

(A and B) After other synaptic responses were blocked with a solution containing receptor antagonists (picrotoxin, prazosin, DNQX, MK-801, CGP 56999a), the outward current that remained was completely inhibited by the D2-like receptor antagonist sulpiride (50–150 nM). This D2-type receptor-mediated IPSC is represented on the left side of trace (A) and expanded in trace (B). Iontophoretic application of dopamine (+190 nA; 100 ms) elicited a similar outward current that was also blocked by sulpiride (right trace of trace [A]).

(C) Under control conditions in the loose cell-attached patch configuration, stimulation of dopamine neurons produced an approximately 1 s pause in the pacemaker firing pattern that these cells exhibit in vitro (top; four sweeps overlaid). This pause was eliminated by the D2 receptor antagonist sulpiride (150 nM; bottom), suggesting that D2 receptors are responsible for the inhibition of cell firing.

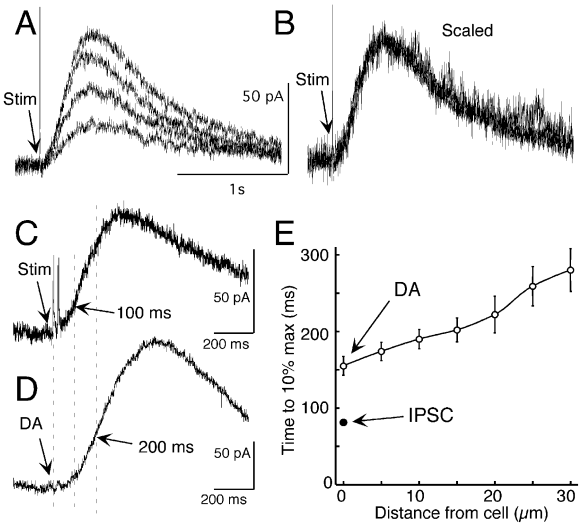

Figure 2. Evidence against the Role of Volume Transmission in the Dopamine IPSC

(A and B) The kinetics of the dopamine IPSC were not different when the amplitude of the current was changed by altering the stimulus intensity (A), as can be observed when the same traces are scaled to their maximums (B).

(C and D) The dopamine IPSC began within 80 ms of the stimulation (C), while the most rapid dopamine iontophoretic responses (20 ms pulse) were consistently 50–100 ms slower to activate than the IPSC (D).

(E) The iontophoretic pipette was moved away from the cell in 5 μm increments to observe the time course of dopamine diffusion. The activation of the IPSC (n = 8) was consistently faster than the fastest iontophoretic response (n = 5), suggesting that physiologically released dopamine acts locally and not in a paracrine manner consistent with volume transmission.

at 1 min intervals. The consequence of evoked dopamine release on cell excitability was tested using the loose cell-attached patch configuration. Dopamine neurons in vitro typically fire in a regular, pacemaker pattern at 1–5 Hz. Electrical stimulation produced a pause in this firing that lasted approximately 1 s. This pause was eliminated by the D2 dopamine receptor antagonist sulpiride (50–150 nM; Figure 1C). Thus, dopamine released by a train of action potentials is capable of affecting spontaneous activity.

Iontophoretic application of dopamine produced an outward current that was similar to the IPSC, although it was often larger and longer lasting (Figure 1A). This outward current was used as a postsynaptic control throughout the investigation to assay dopamine receptor function.

Varying the stimulation intensity (Figures 2A and 2B) or the number of stimuli (data not shown) did not change the kinetics of the IPSC. There was also no difference in time constants of rise and fall between large (rise, 139 ± 10 ms; fall, 291 ± 42 ms) and small (rise, 131 ± 24 ms; fall, 293 ± 33 ms) D2 receptor-dependent synaptic currents. The onset of the current was defined as the time between stimulation and when the current reached 10% of the maximum. This measure was analyzed to determine if dopamine was acting locally (i.e., synaptically) or in a more diffuse, paracrine manner. The synaptic current regularly reached 10% of the maximum 80 ms after stimulation (Figure 2C). When iontophoretic application of dopamine (20 ms pulse) was applied from as close to the cell body as could be visualized, the time that was required to reach 10% of the maximum was consistently 120–170 ms, approximately 70 ms slower than the synaptic response (Figure 2D). The onset of the D2 receptor current that was induced by iontophoretic application at various distances from the cell (0–30 μm) indicated that dopamine diffused at an apparent rate of approximately 250 μm/s (Figure 2E). These data suggest that the rise in dopamine concentration at the receptors is rapid relative to the kinetics of the potassium conductance underlying the IPSC and that dopamine is acting on D2 receptors in close proximity to the release site.

Since dopamine is a known substrate for all monoamine uptake transporters, the nonspecific uptake blocker cocaine was used to determine the role of uptake on the IPSC. The kinetics of the IPSC were dramatically slowed by bath perfusion of cocaine (Figure 3). Cocaine (1–10 μM) caused a concentration-dependent increase in amplitude and duration (Figures 3A–3C), indicating that dopamine uptake is normally the rate-limiting step in the termination of the IPSC. The current induced by exogenously (iontophoretically) applied dopamine

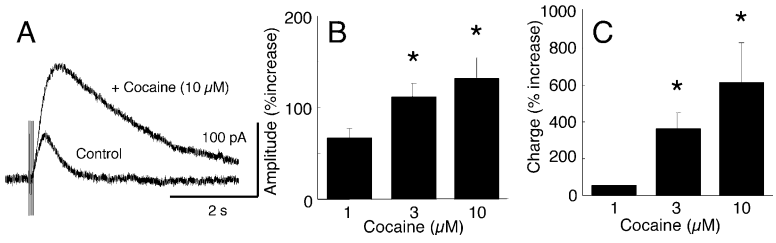

Figure 3. The Dopamine IPSC Is Not Mediated through the Dopamine Transporter

(A) Cocaine (1–10 μM) increased the amplitude and duration of the IPSC, suggesting that dopamine is not released through transporter reversal.

(B and C) Blockade of transporters dose-dependently increased the IPSC peak amplitude (B) and area under the curve (C), suggesting that uptake mediated via monoamine uptake transporters is a critical factor controlling the shape and duration of the IPSC. $p < 0.001$ on ANOVA [(C); $F(3, 30) = 38.6$] or nonparametric ANOVA on ranks (B). Dunnett's (C) or Dunn's (B) post hoc tests revealed statistically significant ($p < 0.05$) changes in both measures for 3 and 10 μM cocaine (*) when compared to predrug control.

was also prolonged by cocaine, as has been reported previously (data not shown; Lacey et al., 1990).

Dopamine Is Released by Exocytosis

Perfusion of calcium-free buffer (plus 0.5 mM EGTA) reversibly eliminated the IPSC without decreasing the iontophoretic response, consistent with a vesicular mechanism of release (Figures 4A and 4B). The IPSC was dependent on activation of more than one voltage-sensitive calcium channel, as the N type channel blocker ω-conotoxin GVIA reduced the IPSC, while cadmium (300 μM) completely blocked the IPSC (Figure 4C; $n = 4$). Nimodipine (3 μM, $n = 4$; 10 μM, $n = 3$) had no effect on the amplitude of the IPSC. These toxins had no effect on the iontophoretic response to dopamine (data not shown). The sensitivity of the IPSC to these toxins could be predicted based on the presence of the calcium currents previously measured in acutely isolated dopamine cells (Cardozo and Bean, 1995). The IPSC was eliminated by treatment of slices with the vesicular monoamine transport inhibitor reserpine (1 μM; Figures 5A–5C) as well as the vesicular H^+-ATPase inhibitor concanamycin (5 μM; Figure 5C). The IPSC was also reversibly eliminated by the voltage-gated sodium channel blocker tetrodotoxin (500 nM; data not shown; $n = 5$), suggesting that neurotransmitter release was dependent on presynaptic depolarization. Taken together, these data suggest that depolarization of dopamine cells activates calcium influx through voltage-sensitive channels, releasing dopamine from somatodendritic vesicular stores to act on dopamine autoreceptors.

The Molecular Components of the IPSC

Although sensitivity to sulpiride suggested that the IPSC was mediated by dopamine, it was possible that noradrenaline or some other unknown transmitter could also activate dopamine receptors and be responsible for the IPSC. Dopamine-deficient (DD) mice were used to address this possibility. DD mice were engineered to lack dopamine (only in dopaminergic cells) and have normal levels of noradrenaline (Zhou and Palmiter, 1995). Although dopamine neurons in these animals have normal sensitivity to D2 receptor agonists (Paladini et al., 2003), no D2 receptor-mediated IPSCs were observed under control conditions in slices from DD mice (Figures 6A and 6C). When the stores of dopamine were increased by superfusing the catecholamine precursor L-3,4-dihydroxyphenylalanine (L-DOPA) (50 μM; 5 min), a sulpiride-sensitive IPSC was detected that increased in amplitude over 15–20 min (Figures 6A and 6C). Thus, dopamine that was released by dopamine cells was responsible for the IPSC. In slices from non-DD littermates, L-DOPA also caused a significant increase in the amplitude of the IPSC (Figures 6B and 6C). This suggests that the vesicular content of dopamine is labile, as has been shown previously (Pothos et al., 1998; Pothos, 2002).

Since activation of a GIRK channel conductance was the most likely candidate for the ionic basis of the IPSC (Kim et al., 1995), we sought to determine the specific potassium channel responsible with potassium channel knockout (KO) mice. Midbrain dopamine cells express mRNA for GIRK1-4 and most prominently GIRK2/Kir3.2 and GIRK3/Kir3.3 channel subunits (Davila et al., 2003). Dopamine receptor-mediated IPSCs (Figure 7A) and dopamine iontophoretic responses (Figure 7B) that were observed in slices from mice lacking GIRK3 subunits were similar to those of wild-type animals. However, virtually no dopamine IPSC was detected in slices from mice lacking GIRK2 channel subunits (Figure 7C), and iontophoresis of dopamine never elicited a current larger than 20 pA (Figure 7D). This suggests that channels containing GIRK2 subunits are responsible for most of the dopamine receptor-mediated synaptic current.

Dopamine cells express both D2 and D3 dopamine

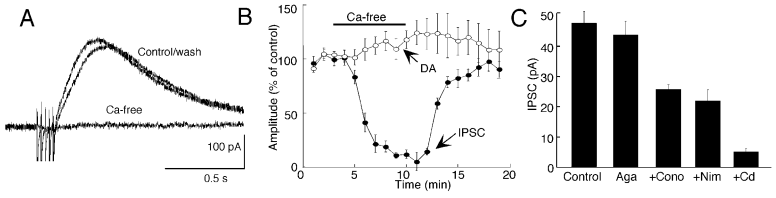

Figure 4. The IPSC is Calcium Dependent and Therefore Likely Vesicular

(A and B) Both a sample trace and summary data ($n = 6$) demonstrate that application of calcium-free perfusate (plus 0.5 mM EGTA) reversibly eliminated the dopamine IPSC (closed circles) without producing a decrease in the iontophoretic response (open circles).

(C) Stepwise application of voltage-sensitive calcium channel blockers ω-agatoxin IVA (100–200 nM; P/Q type), ω-conotoxin GVIA (1–6 μM; N type), nimodipine (3 μM; L type), and cadmium (300 μM; all types) suggested that activation of N type and another type of voltage-sensitive calcium channel is necessary for the IPSC ($n = 4$).

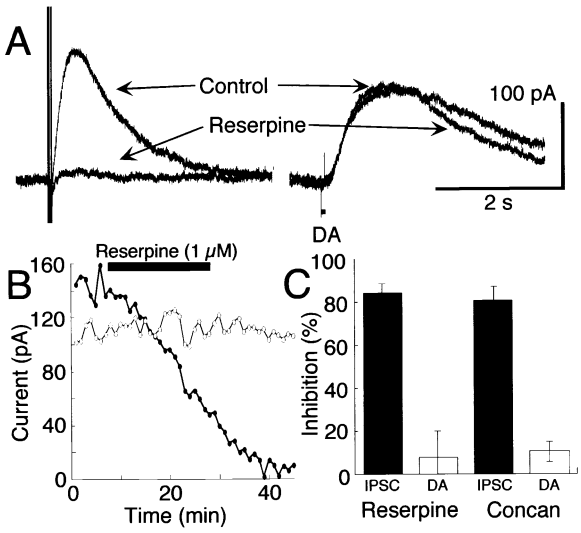

Figure 5. Depletion of Vesicles Eliminates the IPSC

(A) Depletion of catecholamine neurotransmitter vesicles with reserpine (1 μM; 20 min) eliminated the IPSC (left), while the postsynaptic response did not change, evidenced by the current elicited by iontophoretic application of dopamine at the same time points (right).

(B) The time course of the same experiment shows the decline in IPSC amplitude (closed circles) over the 20 min application of reserpine, while the amplitude of the iontophoretic current (open circles) remained unchanged.

(C) Summarized results obtained with reserpine and the vesicular H^+-ATPase inhibitor concanamycin (5 μM) indicate $p < 0.008$ for the difference between change in synaptic versus iontophoretic response for reserpine. $p < 0.001$ for concanamycin.

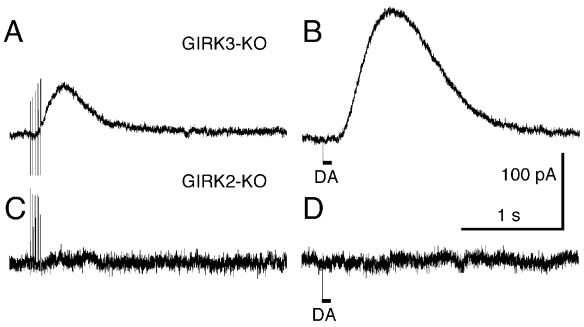

Figure 7. Channels Containing GIRK2 Subunits Underlie the Dopamine IPSC

(A and B) Representative traces from GIRK3 KO mouse brain slices indicated a wild-type-like dopamine IPSC (A) and outward current induced by iontophoretic dopamine (B).

(C and D) However, in slices from GIRK2 KO animals, virtually no dopamine IPSC (C) or response to iontophoretic dopamine (D) was detected. This result indicates that channels containing GIRK2 subunits are largely responsible for the IPSC in wild-type animals. IPSC peak amplitudes: 12.5 ± 2.03 pA, n = 12 for GIRK2 KOs; 59.7 ± 10.2, n = 7 for GIRK3 KOs.

receptor subtypes, which are difficult to isolate pharmacologically due to the absence of highly selective ligands. Therefore, the physiological role of D3 receptors has not been determined and has been the subject of debate. D3 receptors activate GIRK channels in mammalian cell lines (Kuzhikandathil et al., 1998) but not in acutely dissociated dopamine neurons from *D2r* (D2 receptor gene) KO mice (Davila et al., 2003). To determine if D3 receptors contribute to the synaptic response, we conducted experiments in slices from *D2r* KO and *D3r* KO mice (Kelly et al., 1997; Accili et al., 1996). After blockade of $GABA_B$ receptors with CGP 35348 (30–100 μM), stimulation of slices from *D3r* KO animals produced a dopamine-mediated IPSC, similar to data obtained in slices from wild-type animals (Figure 8A). However, no IPSC could be detected in *D2r* KO animals, and no dopamine iontophoretic response was detected (data not shown). This lack of effect was not due to the absence of GIRK channels in the *D2r* KO animals, since $GABA_B$ receptor-mediated IPSCs were still observed (Figure 8B; Cruz et al., 2004). This suggests that D2 receptors mediate the dopamine IPSC and that any component mediated solely through D3 receptors is below the limit of detection.

Discussion

We have identified an IPSC mediated through exocytosis of the neurotransmitter dopamine in midbrain dopamine cells. Somatodendritic dopamine release was first hypothesized three decades ago (Bjorklund and Lindvall, 1975; Geffen et al., 1976; Groves et al., 1975) and was later confirmed through microdialysis and electrochemical techniques (Hoffman and Gerhardt, 1999; Kalivas and Duffy, 1991; Rice et al., 1997). The inability to detect dopamine-mediated synaptic currents in pro-

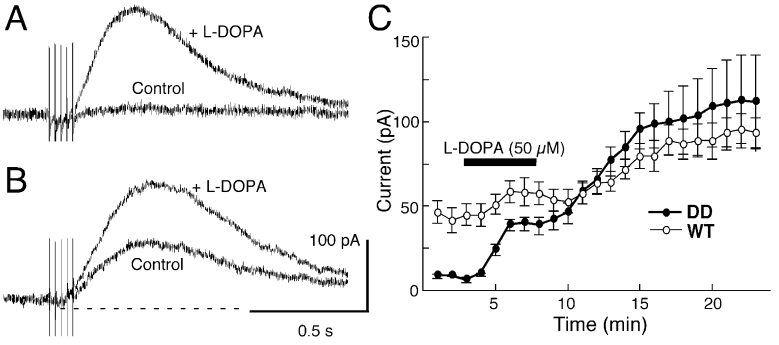

Figure 6. The IPSC Is Mediated by the Neurotransmitter Dopamine

(A) In mutant mice lacking dopamine in midbrain dopamine cells, no dopamine-mediated IPSC could be detected under control conditions. Bath perfusion of the dopamine precursor L-DOPA (50 μM for 5 min) rescued the IPSC, suggesting that dopamine is the neurotransmitter responsible for the current.

(B) Dopamine synaptic currents were detected under control conditions in slices from non-dopamine-deficient littermates, and bath application of L-DOPA again increased the amplitude of the IPSC, presumably through filling of synaptic vesicles with dopamine.

(C) The time course indicates that, although the DD mice do not exhibit dopamine synaptic currents at first, only minutes are needed after application of L-DOPA before the IPSC resembles that of wild-type mice.

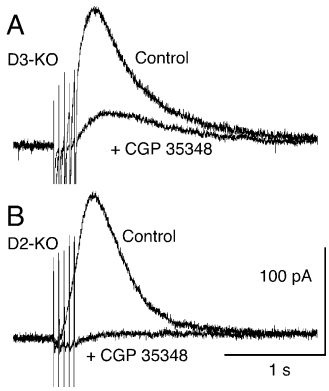

Figure 8. D2 Receptors Mediate the Dopamine IPSC

(A) In *D3r* knockout mice, dopamine cells exhibited an IPSC similar to wild-type, including a sulpiride-sensitive component revealed by blockade of $GABA_B$ receptors with CGP 35348 (30 μM).

(B) In slices from *D2r* knockout mice, however, the $GABA_B$ receptor-mediated IPSC remained, while no detectable dopamine IPSC ($p < 0.001$ versus *D3r* KO) or iontophoretic response (data not shown) was detectable. The presence of a $GABA_B$ IPSC indicates that GIRK channels were intact in the *D2r* knockout animals. IPSC peak amplitudes: 6.88 $\pm$ 2.36 pA, n = 9 for D2 KOs; 29.4 $\pm$ 4.57, n = 9 for D3 KOs.

jection areas and in cell body regions has lead to the assertion that dopamine acts in a paracrine fashion, diffusing over some distance to reach dopamine receptors (volume transmission; Fuxe and Agnati, 1991; Rice et al., 1997; Rice, 2000). The present report suggests that synaptic dopamine transmission directly regulates cell excitability, is mediated through exocytosis, and does not depend on volume transmission.

Reuptake Regulates the Duration of Transmission

The dramatic effects of cocaine on the kinetics of the IPSC suggest that transporter activation is the limiting factor controlling the peak and duration of the effect. This is somewhat surprising given previous suggestions that dopamine overflow in the midbrain is not as tightly regulated by transporter activity as that in the striatum (Rice, 2000). Our inability to change the time course of the IPSC by varying stimulus intensity is a further indication of the tight regulation of extracellular dopamine by uptake transporters. Interestingly, despite repeated efforts to show otherwise, iontophoretic application of dopamine consistently produced currents that activated more slowly than the IPSC. Given that activation of G protein signaling typically requires 30–150 ms, a faster rise of dopamine is below the limit of detection. Cocaine increased the amplitude *and* duration of the IPSC, while higher stimulus intensities only augmented the amplitude without affecting the time course, suggesting that diffusion of dopamine over a distance contributes to the physiological response only when transporters are overwhelmed or blocked. So, while forced volume transmission (subsequent to cocaine administration or with an iontophoretic application of dopamine) is possible and may play a role in other forms of dopamine transmission, it does not appear to contribute to the synaptic response that was seen subsequent to the stimulation protocol used in this investigation. Stronger stimuli may be necessary to overwhelm uptake transporters and produce detectable dopamine overflow into widespread areas, which would help to explain the discrepancies between the present work and previously published observations.

Glutamate-Evoked Dopamine Release

Glutamate release produces biphasic effects on dopamine neurons, mediating fast excitation via NMDA and AMPA receptors followed by slow inhibition through metabotropic receptors (Fiorillo and Williams, 1998). Our data suggest that dopamine release dampens cell excitability on a timescale that is slower than ionotropic glutamatergic excitation but faster than metabotropic glutamate receptor inhibition (Fiorillo and Williams, 1998). Administration of the D2-like receptor antagonist haloperidol, either systemically or directly into the midbrain, increases dopamine cell firing rates (Bunney et al., 1973; Groves et al., 1975). That this effect persists when the connections between the midbrain and basal ganglia have been severed suggests that under physiological conditions dendrodendritic dopamine transmission exerts a powerful influence on the firing properties of these neurons (Pucak and Grace, 1994). Burst firing of dopamine neurons in vivo typically consists of from two to six action potentials fired at 6–15 Hz (Grace and Bunney, 1984), meaning that a typical burst lasts 200–500 ms. The dopamine IPSC that is described here may be temporally positioned to contribute to the termination of burst firing, concluding a series of action potentials and producing the pause typically seen in vivo following a burst. Dopamine cell bursting in vivo is produced by glutamate input in response to environmental cues such as an unexpected reward (reviewed by Schultz, 2002) and is associated with a large increase in terminal dopamine release (Gonon, 1988). This glutamate-driven excitation produces simultaneous burst firing of many dopamine neurons (Schultz, 2002), an effect that may be reasonably approximated in vitro by the stimulation protocol used in the present investigation. Stimulation of dopamine neurons produced a sulpiride-sensitive pause in the tonic, pacemaker firing that these cells exhibit in vitro (Figure 1C). Thus, in addition to established effects in terminal regions, midbrain D2 receptor activation may be integral to normal neuronal coding in response to natural rewards and associated conditioned stimuli.

Inhibition of Dopamine Neurons

The identification of a dopamine receptor-mediated IPSC brings the total number of known neurotransmitters that activate synaptic potassium currents in dopamine cells to three, along with GABA ($GABA_B-$; Johnson and North, 1992) and glutamate (metabotropic glutamate receptors; Fiorillo and Williams, 1998). Fitting the rise and fall of the IPSC curve reveals that the onset and termination of the $GABA_B$ IPSC is approximately 2-fold faster than the dopamine IPSC (mean rise time, 74 versus 135 ms; mean decay time, 188 versus 292 ms; data not shown). As reported in the rat, the metabotropic glutamate IPSC is the slowest and most variable of the three, likely because it requires phosphoinositide hydrolysis (Pin and Duvoisin, 1995) and the release of calcium from intracellular stores to activate an apamin-sensitive

conductance (Fiorillo and Williams, 1998). The explanation for the differences in time course between the dopamine and $GABA_B$ receptor responses is not immediately evident, as both are mediated via GIRK channels. It has been reported that activation and deactivation of GIRK conductance in one cell mediated through two different receptors depends on agonist unbinding and can vary in time course by up to an order of magnitude (Benians et al., 2003; Sodickson and Bean, 1998). Alternatively, it could be that the axodendritic $GABA_B$ synapses are better equipped with machinery for neurotransmitter release and sensing or G protein signaling than dendrodendritic dopamine synapses.

The present investigation suggests that D2 receptor activation is one of several mechanisms that inhibit dopamine cells on a millisecond timescale. The results indicate that there is a labile, readily releasable vesicular pool of dopamine molecules that can be rapidly enhanced by exogenous application of L-DOPA or decreased with agents that disrupt dopamine entry into vesicles. Acute administration of amphetamine depletes vesicular stores, and other addictive and therapeutic agents, such as cocaine, methylphenidate, and some antidepressants, could affect vesicular dopamine stores and thus the level of dopamine autoinhibition. Thus, determining what alterations occur in this system under pathological conditions such as Parkinson's disease, schizophrenia, attention deficit/hyperactivity disorder, and substance abuse could potentially aid in identifying new therapeutic targets for these conditions.

Experimental Procedures

Animals

All experiments were approved by the Institutional Animal Care and Use Committee, and facilities were accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. Male and female wild-type C57BL/6 mice (Jackson Laboratories, Bar Harbor, ME) more than 5 weeks old were used in experiments. Creation of *D2r* (Kelly et al., 1997) and *D3r* (Accili et al., 1996) KO mice, *Kir3.2* (GIRK2; Signorini et al., 1997) and *Kir3.3* (GIRK3; Torrecilla et al., 2002) KO mice, and DD mice ($TH^{-/-}D\beta H^{+/TH}$; Zhou and Palmiter, 1995) has been described previously. *D2r* and *D3r* KO mice were backcrossed onto the C57BL/6J line for 20 (N20) and 10 (N10) generations, respectively. Mice were maintained and, on the day of the experiments, sacrificed in accordance with accepted animal care and use protocols.

Slice Preparation and Recording

Horizontal slices (220 μm) of the ventral midbrain were prepared as described previously (Williams et al., 1984). Slices were maintained at 35°C and perfused at a rate of 1.5 ml/min with a solution containing 126 mM NaCl, 2.5 mM KCl, 1.2 mM $MgCl_2$, 2.4 mM $CaCl_2$, 1.4 mM NaH_2PO_4, 25 mM $NaHCO_3$, and 11 mM D-glucose. Using DIC optics and infrared illumination, dopamine cells in the VTA and the SN pars compacta were identified visually by their location in relation to the medial terminal nucleus of the accessory optic tract. Physiological identification was based on the presence of spontaneous pacemaker firing at 1–5 Hz, a large hyperpolarization-induced I_H current, and sensitivity to iontophoretically applied dopamine. Pipettes for whole-cell patch clamp (1.4–2.0 MΩ) were filled with a solution containing 115 mM K-methylsulfate, 20 mM NaCl, 1.5 mM $MgCl_2$, 10 mM BAPTA, 2 mM ATP, 0.2 mM GTP, and 10 mM phosphocreatine, pH 7.35, 268–275 mOsm. Experiments were performed in whole-cell patch-clamp configuration with the cells voltage clamped at −55 to −60 mV with an Axopatch 1D amplifier (Axon instruments, Foster City, CA). Series resistance ranged from 2.0 to 15 MΩ, was monitored throughout the experiment, and was compensated by 80%. Pipette filling solution used in the cell-attached configuration lacked ATP, GTP, and phosphocreatine and was adjusted to pH 7.40, 295 mOsm to deter membrane rupturing.

Synaptic currents mediated by dopamine were isolated using the following mixture of receptor blockers: picrotoxin (100 μM), prazosin (100 nM), MK-801 (10 μM), and in most experiments (6,7-dinitroquinoxaline-2,3(1H,4H)-dione) (DNQX) (10 μM) and CGP 56999a or CGP 35348 (100 nM and 30–100 μM, respectively). Metabotropic glutamate receptors were not regularly blocked, as the antagonist (S)-α-methyl-4-carboxyphenylglycine (MCPG) (1 mM) had no effect on the dopamine IPSC (data not shown; $n = 8$). Electrical stimulation was performed via a bipolar platinum electrode with tip separation of approximately 200 μm, placed in the slice 50–100 μm caudal to the cell being monitored. Synaptic currents mediated by dopamine could be detected in most cells using from one to ten pulses (500 μs duration) applied at 40 Hz once every 60 s. Iontophoretic pipettes were pulled from thin-wall microelectrodes (impedance approximately 100 MΩ), filled with dopamine (1 M), and placed 0–30 μm from the cell. A holding current of approximately −3.0 nA was applied to prevent passive leakage of dopamine, and dopamine was ejected as a cation with a single pulse (50–190 nA; 20–100 ms) once every 60 s.

Drugs

Dopamine, prazosin, MK-801, picrotoxin, reserpine, nimodipine, and L-DOPA were obtained from Sigma (St. Louis, MO). Baclofen, sulpiride, and tetrodotoxin were from Research Biochemicals International (Natick, MA). CGP 56999a and CGP 35348 were generous gifts from Novartis Pharmaceuticals (Basel, Switzerland). ω-conotoxin GVIA and ω-agatoxin IVA were obtained from Alomone Labs (Jerusalem, Israel). DNQX and MCPG were from Tocris Cookson (St. Louis, MO). Concanamycin was from Fluka (St. Gallen, Switzerland).

Data Analysis

Data are expressed wherever possible as arithmetic mean ± SEM. Percent change in current caused by application of a drug was determined with the equation 100% × [(current after equilibrium had been reached/the average of the last three predrug data points) − 1]. All analytical statistics were performed with Sigma Stat 2.03.0 (SPSS Inc., Chicago, IL). Analysis of variance (ANOVA) was used in the experiments involving cocaine, although in one case failure of normality necessitated nonparametric analysis of ranks. In some cases, simple comparisons were made with paired (within cells) and unpaired Student's t tests, and statistical significance was defined as $p < 0.05$.

Acknowledgments

We are grateful for our collaborators at the University of Washington: Richard Palmiter, Bethany Sotak, and Siobhan Robinson, who made possible the DD mouse experiments. We would also like to thank Heather Drought and Katherine Suchland for their technical assistance; and Malcolm Low, Susan Ingram, and Craig Jahr for helpful suggestions. This work was supported by NIH grants DA16467 (M.J.B); DA4523 (J.T.W); DK7680 (R.H. Goodman); MH67497, MH66360, and DA12062 (D.K.G); and MH61933 (K.W.).

Received: February 13, 2004
Revised: April 23, 2004
Accepted: May 18, 2004
Published: June 23, 2004

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