Novel Ca^{2+} Dependence and Time Course of Somatodendritic Dopamine Release: Substantia Nigra versus Striatum

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Somatodendritic release of dopamine (DA) in midbrain represents a novel form of intercellular signaling that inherently differs from classic axon-terminal release. Here we report marked differences in the Ca^{2+} dependence and time course of stimulated increases in extracellular DA concentration ($[DA]_o$) between the substantia nigra pars compacta (SNc) and striatum. Evoked $[DA]_o$ was monitored with carbon-fiber microelectrodes and fast-scan cyclic voltammetry in brain slices. In striatum, pulse-train stimulation (10 Hz, 30 pulses) failed to evoke detectable $[DA]_o$ in 0 or 0.5 mM Ca^{2+} but elicited robust release in 1.5 mM Ca^{2+}. Release increased progressively in 2.0 and 2.4 mM Ca^{2+}. In sharp contrast, evoked $[DA]_o$ in SNc was nearly half-maximal in 0 mM Ca^{2+} and increased significantly in 0.5 mM Ca^{2+}. Surprisingly, somatodendritic release was maximal in 1.5 mM Ca^{2+}, with no change in 2.0 or 2.4 mM Ca^{2+}. Additionally, after single-pulse stimulation, evoked $[DA]_o$ in striatum reached a maximum (t_{max}) in <200 msec, whereas in SNc, $[DA]_o$ continued to rise for 2–3 sec. Similarly, the time for $[DA]_o$ to decay to 50% of maximum (t_{50}) was 12-fold longer in SNc than striatum. A delayed t_{max} in SNc compared with striatum persisted when DA uptake was inhibited by GBR-12909 and D_2 autoreceptors were blocked by sulpiride, although these agents eliminated the difference in t_{50}. Together, these data implicate different release mechanisms in striatum and SNc, with minimal Ca^{2+} required to trigger prolonged DA release in SNc. Coupled with limited uptake, prolonged somatodendritic release would facilitate DA-mediated volume transmission in midbrain.

Key words: calcium; dopamine; dopamine transporter; substantia nigra pars compacta; voltammetry; volume transmission; synaptic transmission

Dopamine (DA) neurons of the substantia nigra pars compacta (SNc) send axon projections to the dorsal striatum via the median forebrain bundle (Fallon et al., 1978). Importantly, these midbrain cells release DA from somata and dendrites, known as somatodendritic release (Geffen et al., 1976; Nieoullon et al., 1977; Rice et al., 1994; Jaffe et al., 1998), as well as from axon terminals in striatum. Both somatodendritic and terminal release are critical for the control of movement mediated by the basal ganglia (Robertson and Robertson, 1989; Timmerman and Abercrombie, 1996; Crocker, 1997).

Logic might dictate that somatodendritic release is mediated by a novel mechanism, given the novel source of release. However, no known characteristics contradict the original proposal by Geffen et al. (1976) that release in SNc is vesicular and mediated by exocytosis, as it is in striatum. Indeed, somatodendritic DA release is depolarization and Ca^{2+}dependent (Geffen et al., 1976; Cheramy et al., 1981; Rice et al., 1994, 1997) and reserpine-sensitive (Elverfors and Nissbrandt, 1991; Rice et al., 1994; Heeringa and Abercrombie, 1995). Other pharmacological agents, including DA-releasing drugs such as amphetamine, DA transport inhibitors, and D_2 autoreceptor antagonists, cause parallel increases in extracellular DA concentration ($[DA]_o$) in SNc and striatum, although to a lesser extent in SNc (Santiago and Westerink, 1992; Heeringa and Abercrombie, 1995; Cragg and Greenfield, 1997; Cragg et al., 1997; Hoffman and Gerhardt, 1999).

In particular, evidence for the Ca^{2+} dependence of release is often taken as confirmatory of vesicular release, because Ca^{2+} entry is required for exocytosis (Douglas and Rubin, 1963; Simon and Llinás, 1985; Burgoyne and Morgan, 1995; Catterall, 1999). Influx of Ca^{2+} promotes vesicle fusion via molecular machinery that includes the vesicle membrane proteins synaptobrevin and synaptotagmin, and the presynaptic membrane proteins syntaxin and SNAP-25 (synaptosomal-associated protein of 25 kDa) (Jahn and Südhof, 1994; Catterall, 1999). Moreover, the amount of transmitter released depends on extracellular Ca^{2+} concentration ($[Ca^{2+}]_o$), with increased Ca^{2+} entry and enhanced release in elevated $[Ca^{2+}]_o$ (Dodge and Rahamimoff, 1967). Consistent with classical exocytosis, axon-terminal DA release in striatum increases with increasing $[Ca^{2+}]_o$ both *in vivo* (Moghaddam and Bunney, 1989) and *in vitro* (Chen et al., 2001).

Somatodendritic DA release in SNc also requires Ca^{2+}. In contrast to striatal release, however, evoked DA release in SNc persists in low-Ca^{2+} media (Hoffman and Gerhardt, 1999). Indeed, prolonged incubation in Ca^{2+}-free media plus EGTA is required to inhibit release by 90% (Rice et al., 1994, 1997), suggesting a potential difference in the Ca^{2+} dependence of somatodendritic versus terminal release.

Despite apparently strong pharmacological support for similar mechanisms of somatodendritic and axon terminal release, the discrepancy in Ca^{2+} dependence indicated that additional investigation was necessary. To this end, we evaluated $[DA]_o$ evoked during pulse-train stimulation in varying $[Ca^{2+}]_o$ in striatum and SNc in guinea pig brain slices. Additionally, real-time monitoring

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of DA release after single-pulse stimulation permitted comparison of the time course of somatodendritic versus terminal release; contributions from differences in DA transporter (DAT) activity and D_2 autoreceptor regulation were also assessed.

MATERIALS AND METHODS

Slice preparation and solutions. Male Hartley guinea pigs (150–250 gm) were deeply anesthetized with 40 mg/kg pentobarbital (intraperitoneally) and decapitated. All animal handling procedures were in accordance with National Institutes of Health guidelines and were approved by the New York University School of Medicine Animal Care and Use Committee. Coronal striatal and midbrain slices (400 μm) were prepared as described previously (Rice et al., 1997; Chen et al., 2001) using a Vibratome (Ted Pella, St. Louis, MO). Slice coordinates for midbrain were between 7.3 and 8.3 mm anterior to the interaural line (Smits et al., 1990). All slices were cut in ice-cold HEPES-buffered artificial CSF (ACSF) containing (in mM): 120 NaCl, 5 KCl, 20 $NaHCO_3$, 6.7 HEPES acid, 3.3 HEPES salt, 2 $CaCl_2$, 2 $MgSO_4$, and 10 glucose (saturated with 95% O_2–5% CO_2). After cutting, slices were bisected and then allowed to recover in HEPES-buffered ACSF for at least 1 hr at room temperature before being transferred to a submersion recording chamber (Warner Instruments, Hamden, CT). Once in the recording chamber, slices were equilibrated for an additional 30 min with ACSF, which contained (in mM): 124 NaCl, 3.7 KCl, 26 $NaHCO_3$, 0, 0.5, 1.5, 2.0, or 2.4 $CaCl_2$, 1.3 $MgSO_4$, 1.3 KH_2PO_4, and 10 glucose (saturated with 95% O_2–5% CO_2). Chamber temperature was maintained at 32°C with a flow rate of 1.2 ml/min. The influence of DA uptake and D_2 autoreceptor activation on the time course of evoked $[DA]_o$ was examined using the selective DAT inhibitor GBR-12909 (0.3 and 2 μM) (Bull et al., 1990; Cragg et al., 1997, 2001) and the D_2 antagonist sulpiride (1 μM) (Cragg and Greenfield, 1997; Chen et al., 2001).

Microelectrodes and voltammetric instrumentation. Carbon-fiber electrodes made from 7–8 μm carbon fibers (type HM, unsized, Courtaulds) were spark-etched to a tip diameter of 2–4 μm (MPB Electrodes; Queen Mary and Westfield College, London, UK). The voltammetric method used for all experiments was fast-scan cyclic voltammetry (FCV). Data were obtained using a Millar Voltammeter (PD Systems International, West Molesey, UK), with data acquisition controlled by Clampex 7.0 software (Axon Instruments, Foster City, CA), which imported voltammograms to a personal computer via a DigiData 1200B analog-to-digital converter board (Axon Instruments). Scan rate for FCV was 800 V/sec, with a sampling interval of 100 msec controlled by an external timing circuit. Scan rage was −0.7 V to +1.3 V (vs Ag/AgCl). Voltammograms were obtained in two-electrode mode, with an Ag/AgCl wire in the recording chamber as the reference electrode. Electrodes were calibrated in the recording chamber at 32°C with 0.5–2 μM DA in all media used in a given experiment (Kume-Kick and Rice, 1998; Chen and Rice, 1999), e.g., ACSF with varying Ca^{2+} concentration and/or containing GBR-12909 plus sulpiride. We have reported previously that DA concentrations are stable in oxygenated ACSF at 32°C in the recording chamber (Kume-Kick and Rice, 1998). Evoked $[DA]_o$ was calculated using post-experiment DA calibration factors (typically 2–3 nA/μM) to convert measured oxidation current to concentration. Although a relatively high level of GBR-12909 (10 μM) can alter electrode sensitivity to DA (Davidson et al., 2000), the lower concentrations used in the present studies had no effect.

Electrical stimulation. Bipolar stimulating electrodes were made from Teflon-coated platinum wire (50 μm bare, 75 μm coated) with tip separation of ~50 μm. The electrode was placed on the slice surface with the carbon-fiber microelectrode positioned between the electrical poles and inserted 50–100 μm into the slice, as described previously (Rice et al., 1997; Chen et al., 2001). Pulse-train (10 Hz, 30 pulses) and single-pulse stimulation were used to evoke DA release. Pulse duration was 100 μsec for trains and 1000 μsec for single pulses; pulse amplitude was 0.4–0.8 mA. With these stimulating electrodes and protocols, evoked DA release in SNc is tetrodotoxin sensitive (data not shown), as it is in striatum (Chen et al., 2001). This contrasts with the tetrodotoxin insensitivity of DA release elicited using larger stimulating electrodes and higher stimulus intensity in our previous studies in midbrain and striatum (Rice et al., 1997).

Experimental design. In striatum, consistent evoked $[DA]_o$ can be elicited with repetitive local stimulation (Bull et al., 1990; Chen et al., 2001). Here, striatal DA release was evoked at 10 min intervals in both pulse-train and single-pulse experiments. For Ca^{2+}-dependence studies in striatum, the third of three consistent evoked increases in $[DA]_o$ was included in data averages for each $[Ca^{2+}]_o$. For initial uptake and autoreceptor studies in striatum, one-pulse control records were obtained, and then GBR-12909 plus sulpiride applied. Maximal effects were seen after 1 hr. For subsequent studies in striatum, slices were preincubated for 1 hr in these agents, and the third of three consistent records was used in data averages; similar results were obtained with both protocols. The contralateral hemisphere served as the control for experiments in which preincubation was used. In SNc, maximal release is seen with the first stimulus and then progressively decreases with repetition (Rice et al., 1997). Thus, for measurements in SNc with varying Ca^{2+}, pulse-train-evoked DA release was obtained in medial SNc in a given $[Ca^{2+}]_o$; a different $[Ca^{2+}]_o$ was tested on the contralateral side. For single-pulse studies with GBR-12909 and sulpiride in SNc, one hemisphere of a given midbrain slice was superfused with the drugs for 1 hr; the contralateral side served as the paired control and was superfused with ACSF for 1 hr before release was elicited.

Drugs and chemicals. Sulpiride, DA, and components of ACSF and HEPES–ASCF were obtained from Sigma (St. Louis, MO); GBR-12909 was from Research Biochemicals (Natick, MA). All solutions were made immediately before use.

Statistical analysis. All data are given as means ± SEM, in which n is the number of slices. Differences in evoked $[DA]_o$ in varying Ca^{2+} were assessed using one-way ANOVA, followed by Kruskal–Wallis *post hoc* analysis of maximum evoked $[DA]_o$. Differences in the time course of increases in $[DA]_o$ between SNc and striatum and after DAT and autoreceptor inhibition in each region were assessed using t test comparisons of time of maximum $[DA]_o$ (t_{max}) of the time after the stimulus at which $[DA]_o$ had decayed to 50% of the maximum (t_{50}).

RESULTS

Under most conditions examined, absolute levels of evoked $[DA]_o$ differed between SNc and striatum. To facilitate comparisons among different conditions between regions, therefore, the data were normalized with respect to a defined control condition for each region (100%). Data are illustrated as percentage of control, with peak $[DA]_o$ for each condition indicated in the text. For analysis of the Ca^{2+} dependence of release, maximum evoked $[DA]_o$ in 1.5 mM Ca^{2+} in striatum or in SNc was considered to be 100% for that region, because that was the concentration in which $[DA]_o$ was maximal in SNc.

Ca^{2+} dependence of evoked DA release in striatum and SNc

In striatum, DA release elicited by a train of 30 pulses delivered at 10 Hz showed a marked dependence on $[Ca^{2+}]_o$ (Fig. 1*A*). Release was below detection limits after 30 min superfusion of ACSF with nominally 0 or 0.5 mM Ca^{2+} and then increased progressively with increasing $[Ca^{2+}]_o$. Peak $[DA]_o$ was 0.65 ± 0.05 μM ($n = 17$) in 1.5 mM Ca^{2+}, 1.10 ± 0.13 μM ($n = 10$) in 2.0 mM Ca^{2+}, and 1.81 ± 0.14 μM ($n = 6$) in 2.4 mM Ca^{2+} (Fig. 1*A*). Each increase in $[DA]_o$ was significantly higher than in the previous Ca^{2+} ($p < 0.001$). Taking maximum evoked $[DA]_o$ in 1.5 mM Ca^{2+} as 100%, increasing Ca^{2+} to 2.0 mM caused an increase in $[DA]_o$ to 170%, with an additional increase to 280% in 2.4 mM $[DA]_o$ (Fig. 2). These data suggest an approximately exponential dependence of DA release from striatal terminals on $[Ca^{2+}]_o$.

Evoked $[DA]_o$ in the SNc showed a dependence on $[Ca^{2+}]_o$ that was opposite to that seen in striatum (Fig. 1*B*). In SNc, DA release was readily detected in nominally 0 mM Ca^{2+}, with an average maximum $[DA]_o$ of 0.30 ± 0.06 μM ($n = 7$) (Fig. 1*B*). Evoked $[DA]_o$ increased significantly when $[Ca^{2+}]_o$ was increased to 0.5 mM Ca^{2+} (0.49 ± 0.05 μM; $n = 8$) and again in 1.5 mM Ca^{2+} (0.78 ± 0.09 μM; $n = 14$; $p < 0.01$ for each increase). Somatodendritic DA release was maximal in 1.5 mM Ca^{2+}, however, with no additional increases in 2.0 or 2.4 mM Ca^{2+} (0.76 ± 0.12 μM in 2.0 mM Ca^{2+}, $n = 8$; 0.79 ± 0.10 μM in 2.4 mM Ca^{2+},

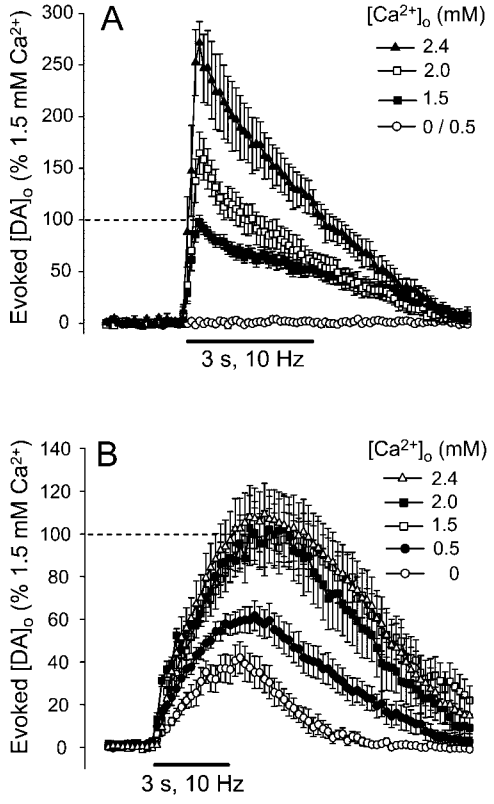

Figure 1. Evoked $[DA]_o$ in the presence of varying $[Ca^{2+}]_o$ in striatum and SNc. Average evoked $[DA]_o$ during pulse-train stimulation (10 Hz, 30 pulses) in striatum (*A*) and SNc (*B*) in 0, 0.5, 1.5, 2.0, and 2.4 mM Ca^{2+}. Maximum $[DA]_o$ in 1.5 mM was taken as 100%. In striatum, peak $[DA]_o$ was significantly increased by each step increase in $[Ca^{2+}]_o$ ($p < 0.001$; 0 and 0.5 mM Ca^{2+}; data from striatum were pooled). In SNc, significant increases were observed between 0 and 0.5 and 1.5 mM Ca^{2+} ($p < 0.01$); however, no additional increases were seen in 2.0 or 2.4 mM Ca^{2+}. Data are means ± SEM ($n = 6$–17). The *dashed lines* indicate 100%, and *solid bars* indicate the stimulation period. Note the difference in time scale between *A* and *B*.

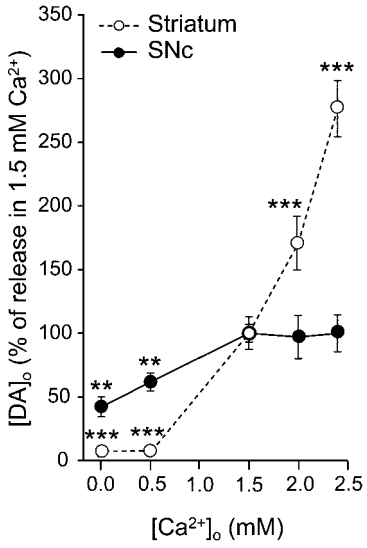

Figure 2. Ca^{2+} dependence of evoked DA release in striatum and SNc. Data were normalized such that 100% is the average maximum evoked $[DA]_o$ during pulse-train stimulation (3 sec, 10 Hz) in 1.5 mM Ca^{2+}. In striatum, evoked $[DA]_o$ increased progressively with increasing Ca^{2+} from 1.5 to 2.4 mM. In contrast, DA release was nearly half-maximal in nominally 0 mM Ca^{2+} in SNc but reached a plateau at 1.5 mM Ca^{2+}. Data are means ± SEM ($n = 6$–17). $^{**}p < 0.01$ and $^{***}p < 0.001$ indicates difference from the response in 1.5 mM Ca^{2+} for each region.

$n = 24$) (Fig. 1*B*). This plateau could be clearly seen when $[DA]_o$ was plotted against $[Ca^{2+}]_o$ (Fig. 2; again taking evoked $[DA]_o$ in 1.5 mM Ca^{2+} as 100%). This suggests that somatodendritic DA release is relatively Ca^{2+} independent beyond a minimal range of $[Ca^{2+}]_o$ required to trigger release.

Because $[DA]_o$ is more strongly limited by DA uptake and D_2 autoreceptor activation in striatum than in SNc (Cragg and Greenfield, 1997; Cragg et al., 1997, 2001; Hoffman and Gerhardt, 1999), low release in 0 mM Ca^{2+} in striatum could appear to be no release if $[DA]_o$ were kept below detection limits by uptake or autoreceptor-mediated inhibition. To test this, we evaluated striatal release evoked by pulse-train stimulation (10 Hz, 30 pulses) in 0 mM Ca^{2+} in the presence of the DAT inhibitor GBR-12909 (2 μM) (Cragg et al., 2001) and the D_2 autoreceptor antagonist sulpiride (1 μM) (Cragg and Greenfield, 1997; Chen et al., 2001). Under these conditions, there was still no detectable increase in evoked $[DA]_o$ in striatum in 0 Ca^{2+} ($n = 5$; data not shown).

Differing time course of evoked $[DA]_o$ in striatum and SNc

A second difference between terminal and somatodendritic DA behavior was indicated by the distinct time course of evoked $[DA]_o$ during pulse-train stimulation in striatum versus SNc. In striatum, $[DA]_o$ rose to a maximum within the first two to five pulses of the train and then decayed during continued stimulation (Fig. 1*A*). In contrast, evoked $[DA]_o$ in the SNc not only increased throughout the stimulus train but also continued to rise for 1–2 sec after the train ended, with the exception of the response in 0 mM Ca^{2+} (Fig. 1*B*). To characterize the time courses of these responses more fully, we used single-pulse stimulation to evoke DA release; 2.4 mM Ca^{2+} was used to ensure reproducible evoked $[DA]_o$ in striatum.

Single-pulse stimulation elicited consistent DA release in both striatum and SNc (Fig. 3). As in pulse-train experiments, the time course of the responses differed markedly between these regions (Fig. 3*A*). Whereas the time of maximal evoked $[DA]_o$ (t_{max}) was <200 msec after stimulus onset in striatum (190 ± 40 msec; $n = 8$), in SNc, evoked $[DA]_o$ did not reach a maximum for 2–3 sec in SNc (2490 ± 460 msec; $n = 9$) ($p < 0.001$ for SNc vs striatum). Importantly, the released substance was clearly identified as DA in both regions by the characteristic DA voltammograms recorded at the response maxima (Fig. 3*B*). The return to baseline was also more rapid in striatum than in SNc, with values for t_{50} (the time after stimulus at which maximal $[DA]_o$ had fallen by 50%) of 540 ± 80 msec ($n = 8$) in striatum and 6400 ± 240 msec ($n = 9$) in SNc ($p < 0.001$; SNc vs striatum).

Like $[DA]_o$ amplitude, as discussed above, the duration of stimulated increases in $[DA]_o$ can also be curtailed by DA uptake and D_2 autoreceptor activation, again with greater efficacy in striatum than in SNc (Cragg and Greenfield, 1997; Cragg et al., 1997, 2001; Jones et al., 1998; Hoffman and Gerhardt, 1999). To address the extent to which these factors might differentially influence time course in the present studies, we examined two concentrations of GBR-12909 (0.3 and 2 μM) (Cragg et al., 1997, 2001) in the presence of a single, supramaximal concentration of the D_2 autoreceptor antagonist sulpiride (1 μM) (Cragg and Greenfield, 1997; Chen et al., 2001). Consistent with the anticipated effects of these drugs, evoked increases in $[DA]_o$ elicited by

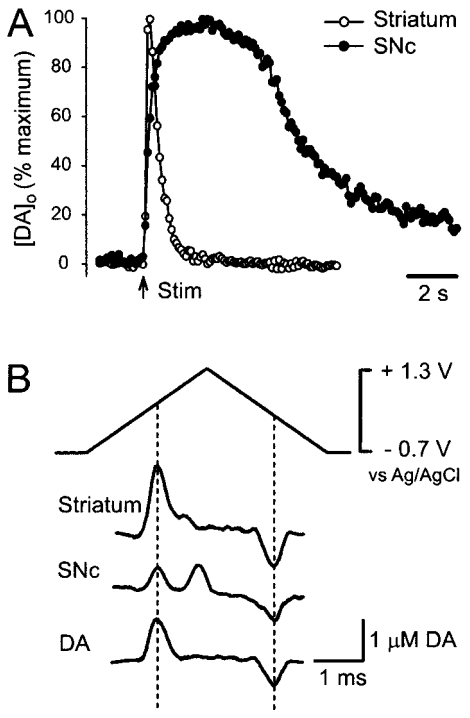

Figure 3. Time course of evoked $[DA]_o$ in striatum and SNc after single-pulse stimulation. *A*, Average evoked $[DA]_o$ by a single pulse (1 msec) in striatum ($n = 8$) and SNc ($n = 9$) (error bars have been omitted for clarity; see Fig. 4). The pattern of release differed significantly, as indicated by the difference in the time of maximum $[DA]_o$ (t_{max}) and time to decay to 50% of maximum (t_{50}) ($p < 0.001$ for both parameters; see Results for details). *B*, DA voltammograms recorded at the time of the maximum evoked $[DA]_o$ in striatum and SNc compared with a 1 μM DA calibration voltammogram; these characteristic voltammograms confirm the identity of the released substance as DA (Rice et al., 1997; Chen et al., 2001).

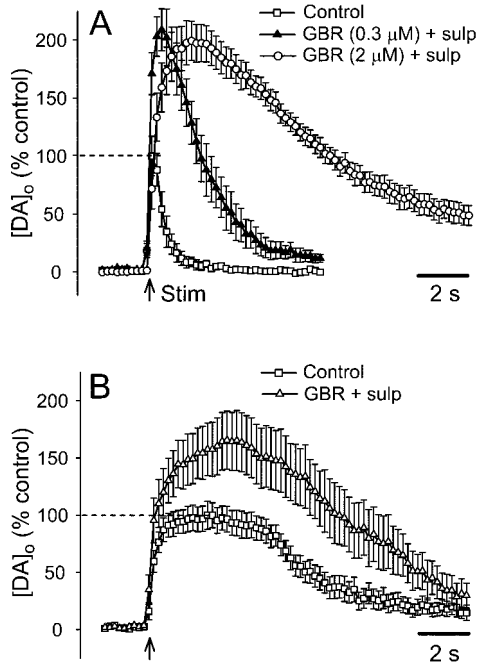

Figure 4. Influence of DAT and D_2 autoreceptor inhibition on evoked $[DA]_o$ in striatum and SNc after single-pulse stimulation. In the presence of GBR-12909 (*GBR*; 0.3 or 2 μM) and sulpiride (*sulp*; 1 μM), average maximum $[DA]_o$ evoked by a single pulse (1 msec) was significantly higher than in controls in striatum (*A*; $p < 0.001$ for both 0.3 and 2 μM GBR-12909; $n = 6–8$) and SNc (*B*; $p < 0.05$ for pooled data from 0.3 and 2 μM GBR-12909; $n = 9$) (see Results for details). Data are means ± SEM; the average maximum $[DA]_o$ in control conditions was taken as 100% for each region, indicated by the *dashed lines*.

single-pulse stimulation were enhanced and prolonged in both striatum (Fig. 4*A*) and SNc (Fig. 4*B*). In striatum, peak $[DA]_o$ increased to ~200% of control in either 0.3 or 2 μM GBR plus sulpiride ($p < 0.001$; $n = 6–8$) (Fig. 4*A*). The enhancement was similar in SNc, with an increase to ~165% of control ($p < 0.05$; $n = 9$); in SNc, $[DA]_o$ records in 0.3 and 2 μM GBR-12909 were indistinguishable and were pooled (Fig. 4*B*).

The changes in time course could best be seen when the curves were normalized, such that maximum $[DA]_o$ for each region and condition was set to 100% (Fig. 5). In striatum, the entire response was prolonged in a dose-dependent manner: in 0.3 μM GBR plus sulpiride, t_{max} increased to 670 ± 90 msec ($n = 6$), with an additional increase to 1700 ± 110 msec ($n = 8$) in 2 μM GBR plus sulpiride (Fig. 5*A*). Similarly, t_{50} increased to 1980 ± 330 msec in 0.3 μM GBR and to 8500 ± 670 msec in 2 μM GBR. All differences in t_{max} and t_{50} between control and each GBR concentration were significant ($p < 0.001$) (Fig. 5*A*). The changes in $[DA]_o$ time course in SNc were much less dramatic than those in striatum, with similar effects in either 300 nM or 2 μM GBR-12909 plus sulpiride, as noted above. Although there was no change in the rising phase of DA records, the falling phase was clearly prolonged (Fig. 5*B*). In fact, the slightly higher t_{max} (3180 ± 410 msec; $n = 9$) did not differ significantly from control ($p > 0.05$), whereas the 30% increase in t_{50} to 8320 ± 550 msec ($n = 9$) was significantly later ($p < 0.01$).

With complete blockade of the DAT and D_2 autoreceptors, DA overflow curves from striatum and SNc became much more similar (compare Figs. 3*A*, 5*C*), primarily because of the much greater changes in DA behavior in striatum. Under these conditions, the initial rising phases of the curves were similar, and the t_{50} values for the two regions were statistically indistinguishable ($p > 0.05$). In SNc, however, $[DA]_o$ continued to increase after the falling phase in striatum had already begun, so that a difference in t_{max} between terminal and somatodendritic release persisted (Fig. 5*C*). The average t_{max} in striatum (1700 msec) was significantly earlier than the time to reach maximum in SNc (3180 msec) ($p < 0.01$). Moreover, $[DA]_o$ in SNc also remained near this maximum for a longer period than in striatum, suggesting more sustained release (Fig. 5*C*).

DISCUSSION

The present comparative studies of the Ca^{2+} dependence and kinetics of DA release in striatum and SNc offer important new insights into both axon terminal and somatodendritic processes. Key results include demonstration of the dependence of evoked $[DA]_o$ in striatum on $[Ca^{2+}]_o$. These data are consistent with previous results showing that basal $[DA]_o$ in DA terminal regions is higher when sampled using microdialysis solutions with elevated Ca^{2+} (Moghaddam and Bunney, 1989). Even more intriguing is the relative independence of evoked $[DA]_o$ in SNc on $[Ca^{2+}]_o$. Together with the longer time to reach maximum $[DA]_o$ in SNc compared with striatum, even in the presence of complete DAT and autoreceptor blockade, these data argue for different underlying mechanisms of axon terminal and somatodendritic release.

Previous studies have questioned classical exocytotic release in

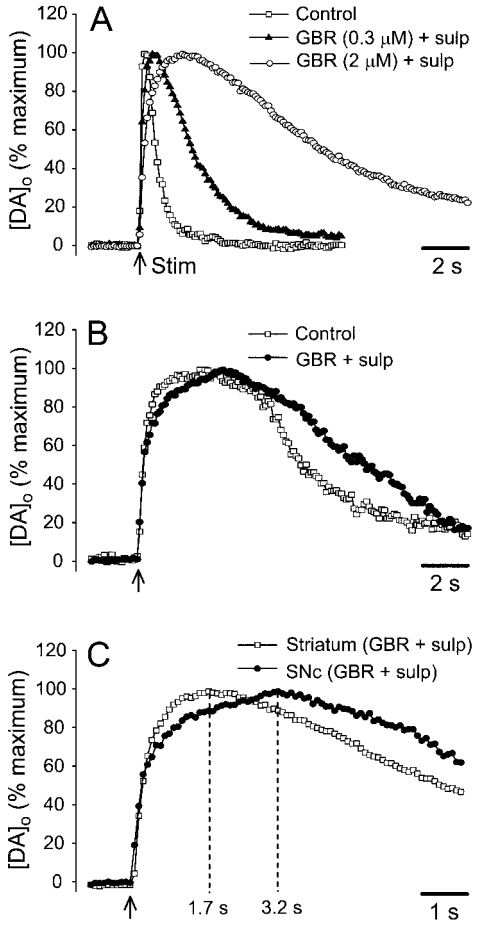

Figure 5. Normalized evoked $[DA]_o$ curves indicate differences in time course between SNc versus striatum. Average $[DA]_o$ evoked by a single pulse in striatum (*A*) and SNc (*B*) in the presence and absence of GBR-12909 (*GBR*) plus sulpiride (*sulp*), with the maximum for each condition normalized to 100%. In striatum, both the t_{max} and t_{50} of evoked $[DA]_o$ increased significantly in GBR-12909 in a concentration-dependent manner ($p < 0.001$ for each step compared with control; $n = 6–8$). Although the t_{50} in SNc was also increased compared with control ($p < 0.01$; $n = 9$ for pooled data from 0.3 and 2 μM GBR-12909), t_{max} was not altered ($p > 0.05$). *C*, When normalized curves for evoked $[DA]_o$ in SNc and striatum in maximally effective GBR-12909 plus sulpiride were superimposed on an expanded time scale, the overall time courses were similar (compare with Fig. 3A), with t_{50} values that did not differ significantly ($p > 0.05$). The time to reach maximum $[DA]_o$, t_{max}, however, remained significantly longer in the SNc ($p < 0.01$); *dashed lines* indicate t_{max} for each region. Data are normalized means; error bars have been omitted for clarity (see Fig. 4).

the substantia nigra on anatomical grounds: synaptic sites available for vesicle fusion are rare. Although dendrodendritic synapses have been described in the SNc (Wilson et al., 1977), these are primarily absent in the SN pars reticulata (SNr) and thus comprise <1% of synaptic input to DA dendrites (Groves and Linder, 1983). Moreover, depolarization-induced DA release can be elicited from the SNr in isolation (Geffen et al., 1976; Rice et al., 1994), suggesting that dendrodendritic synapses are not required for release. Moreover, the number of vesicles in DA somata and dendrites is small. Whereas vesicles are densely localized in identified DA terminals in striatum (Nirenberg et al., 1996a, 1997), there are few vesicles in DA dendrites SNc (Wilson et al., 1977; Groves and Linder 1983; Nirenberg et al., 1996a), implying a limited source for exocytotic release (Nirenberg et al., 1996a). However, somatodendritic DA is stored in saccules of smooth endoplasmic reticulum (Mercer et al., 1978; Wassef et al., 1981), as well as in vesicles (Wilson et al., 1977; Groves and Linder, 1983). Consistent with dual storage sites, the vesicular monoamine transporter VMAT2 is expressed in tuberovesicles that appear to be saccules of smooth endoplasmic reticulum and, less commonly, in vesicles (Nirenberg et al., 1996b). Whether both storage sites contribute to the releasable pool of DA is unknown. Both sites would be susceptible to the DA-depleting actions of reserpine, an irreversible inhibitor of VMAT2, which weakens the argument that reserpine sensitivity indicates vesicular release (Heeringa and Abercrombie, 1995).

Differing Ca^{2+} dependence and kinetics of somatodendritic versus terminal release

In contrast to evoked $[DA]_o$ in striatum, which fell below detectable levels in nominally 0 mM Ca^{2+} even when DA uptake and D_2 autoreceptors were blocked, DA release in SNc persisted in 0 mM Ca^{2+} (Figs. 1, 2), consistent with earlier studies (Hoffman and Gerhardt, 1999). Indeed, evoked $[DA]_o$ was half-maximal in 0 mM Ca^{2+}. Moreover, evoked DA release from synaptic terminals in striatum continued to increase with increasing $[Ca^{2+}]_o$, whereas somatodendritic release was maximal at 1.5 mM Ca^{2+}. These data demonstrate a remarkably limited dynamic range for regulation of somatodendritic release of DA by $[Ca^{2+}]_o$ (Fig. 2). Somatodendritic release does require Ca^{2+}, however, because evoked release can be primarily eliminated by extended incubation in 0 mM Ca^{2+} plus 1 mM EGTA and then restored when Ca^{2+} is added back to the medium (Rice et al., 1994, 1997). Together, these results suggest that a minimal level of Ca^{2+} entry may trigger somatodendritic DA release by a process that is distinct from the classical, Ca^{2+}-dependent exocytotic release from axon terminals in striatum.

Additional evidence for differing mechanisms comes from the prolonged time course of release in SNc compared with striatum (Fig. 3). It is well established that both evoked and exogenously introduced increases in $[DA]_o$ in striatum are enhanced and prolonged when the DAT is inhibited or eliminated (Bull et al., 1990; Kawagoe et al., 1992; Cass et al., 1993; Cragg et al., 1997; Jones et al., 1998) or when D_2 autoreceptors are blocked (Cass and Gerhardt, 1994; Cragg and Greenfield, 1997; Hoffman and Gerhardt 1999). Similar, albeit smaller, increases in evoked $[DA]_o$ in SNc have also been reported after DAT inhibition (Cragg et al., 1997, 2001) or D_2 antagonism (Cragg and Greenfield, 1997; Hoffman and Gerhardt, 1999). The effect of these agents on the time course of evoked $[DA]_o$ in SNc had not been described previously.

As anticipated, the effect of GBR-12909 plus sulpiride on $[DA]_o$ time course was much greater in striatum than SNc (Fig. 4), consistent with the higher expression the DAT in striatum (Donnan et al., 1991; Ciliax et al., 1995; Freed et al., 1995); similar regional comparisons of D_2 autoreceptor expression are not available. The main effect on time course is likely to be from DAT inhibition, however. Indeed, the concentration-dependent effects of GBR-12909 on $[DA]_o$ time course in striatum in the presence of constant sulpiride levels were strikingly similar to the effects of graded DAT loss on single-pulse-evoked DA overflow in wild-type versus heterozygous and homozygous DAT knock-out mice (Jones et al., 1998). Moreover, previous studies have shown that D_2 receptor antagonism does not alter peak $[DA]_o$ evoked with brief (100 msec), high-frequency stimulation in either striatum or SNc (Cragg and Greenfield, 1997; Chen et al., 2001),

reflecting minimal D_2 receptor occupancy and efficacy within this time window (Singer, 1988). Because of known interactions between D_2 activation and DAT activity (Meiergerd et al., 1993; Parsons et al., 1993; Cass and Gerhardt, 1994; Wieczorek and Kruk, 1994; Hoffman et al., 1999), however, sulpiride was included with GBR-12909 in the present studies to prevent possible synergistic interactions.

Whereas differences in DA clearance (t_{50}) between striatum and SNc were eliminated when DA uptake and autoreceptor-mediated suppression of release were inhibited, a significant difference in t_{max} remained (Fig. 5*C*). Because synaptic DA release in striatum presumably occurs within milliseconds of a stimulus (Garris and Wightman, 1995), a t_{max} of >1 sec presumably reflected the time required for diffusion to the electrode from distant sites. The difference in t_{max} between SNc and striatum, however, cannot be explained by differences in diffusion properties. Although the extracellular volume fraction (α) is 50% larger in SNc than in striatum (Cragg et al., 2001), this parameter will influence the amplitude of $[DA]_o$ but not its time course. More importantly, the geometric parameter that governs the apparent diffusion coefficient of a substance in tissue, the tortuosity factor λ, is similar in these regions (Rice and Nicholson, 1991; Cragg et al., 2001) and could not contribute to the twofold difference in t_{max}.

Implications

In combination, the differences in the Ca^{2+} dependence and time course of DA release in SNc and striatum point to an underlying difference in release as well as termination characteristics. The most plausible sources of release differences might be in Ca^{2+} entry and/or regulation or in the releasable pool of DA in somata and dendrites versus axon terminals. Indeed, Wilson and Callaway (2000) showed recently that intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in DA cells of the SNc builds up slowly during a depolarizing step induced by current injection and then persists for a second or more in dendrites and soma when the depolarizing current stops. Time-dependent rather than concentration-dependent Ca^{2+} entry, therefore, might contribute to the relative independence of evoked $[DA]_o$ in SNc on Ca^{2+} above a certain minimal level. Slow clearance of an increase in $[Ca^{2+}]_i$ might also contribute to protracted DA release (Wilson and Callaway, 2000), possibly by facilitating fusion of multiple vesicles rather than the single vesicle assumed in classic synaptic transmission (Triller and Korn, 1982; Stevens, 1993; Matveev and Wang, 2000) or fusion of additional or alternative structures, such as tuberovesicles, which store DA (Mercer et al., 1978; Wassef et al., 1981; Nirenberg et al., 1996b) and conceivably could participate in nonclassical somatodendritic release. Release from multiple compartments, i.e., dendrites and somata (Rice et al., 1994; Jaffe et al., 1998), might also contribute. The behavior of evoked $[DA]_o$ in SNc after single-pulse stimulation is consistent with multiple sources or sites of release. After an initially rapid rise in $[DA]_o$ that is similar in SNc and striatum (Fig. 5*C*), the subsequent increase in $[DA]_o$ in SNc is slower and more sustained, such that t_{max} in SNc occurs after $[DA]_o$ in striatum has already begun to return to baseline (here by diffusion, because uptake was inhibited) (Fig. 5*C*).

Regardless of release mechanism, delayed and prolonged increases in $[DA]_o$ in midbrain after a single stimulus have implications for DA as a mediator of volume transmission. In SNc and SNr, DA receptors on DA cell bodies and dendrites are primarily extrasynaptic (Sesack et al., 1994; Yung et al., 1995; Nirenberg et al., 1996a, 1997). Extrasynaptic D_1 receptors are also found on nondopaminergic terminals in these regions (Yung et al., 1995). Physiological studies suggest that somatodendritically released DA acting at these receptors modulates GABA release from presumed striatonigral GABAergic afferents to SNr (Miyazaki and Lacey, 1998; Radnikow and Misgeld, 1998). Similarly, DA cells in the adjacent ventral tegmental area (VTA) also exhibit somatodendritic release of DA (Iravani et al., 1996; Rice et al., 1997), which can act at extrasynaptic receptors to modulate release of GABA and glutamate in VTA (Cameron and Williams, 1993; Koga and Momiyama, 2000) and which may influence glutamate-mediated plasticity in VTA neurons (Ungless et al., 2001). Thus, to mediate physiological responses, somatodendritically released DA relies on extracellular diffusion to reach its sites of action, which exemplifies volume transmission (Fuxe and Agnati, 1991; Rice, 2000). Prolonged somatodendritic release, as shown in the present studies in SNc, combined with limited DA uptake and D_2 autoreceptor control (Cragg and Greenfield 1997; Cragg et al., 1997, 2001), will facilitate DA-mediated volume transmission in midbrain.

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