Characteristics of Electrically Evoked Somatodendritic Dopamine Release in Substantia Nigra and Ventral Tegmental Area In Vitro

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Rice, M. E., S. J. Cragg, and S. A. Greenfield. Characteristics of electrically evoked somatodendritic dopamine release in substantia nigra and ventral tegmental area in vitro. J. Neurophysiol. 77: 853–862, 1997. Somatodendritic dopamine (DA) release from neurons of the midbrain represents a nonclassical form of neuronal signaling. We assessed characteristics of DA release during electrical stimulation of the substantia nigra pars compacta (SNc) in guinea pig midbrain slices. With the use of parameters optimized for this region, we compared stimulus-induced increases in extracellular DA concentration ([DA]o) in medial and lateral SNc, ventral tegmental area (VTA), and dorsal striatum in vitro. DA release was monitored directly with carbon-fiber microelectrodes and fast-scan cyclic voltammetry. Detection of DA in SNc was confirmed by electrochemical, pharmacological, and anatomic criteria. Voltammograms of the released substance had the same peak potentials as those of DA obtained during in vitro calibration, but different from those of the indoleamine 5-hydroxytryptamine. Similar voltammograms were also obtained in the DA-rich striatum during local electrical stimulation. Contribution from the DA metabolite 3,4-dihydroxyphenylacetic acid to somatodendritic release was negligible, as indicated by the lack of effect of the monoamine oxidase inhibitor pargyline (20 μM) on the signal. Lastly, DA voltammograms could only be elicited in regions that were subsequently determined to be positive for tyrosine hydroxylase immunoreactivity (TH-ir). The frequency dependence of stimulated DA release in SNc was determined over a range of 1–50 Hz, with a constant duration of 10 s. Release was frequency dependent up to 10 Hz, with no further increase at higher frequencies. Stimulation at 10 Hz was used in all subsequent experiments. With this paradigm, DA release in SNc was tetrodotoxin insensitive, but strongly Ca2+-dependent. Stimulated [DA]o in the midbrain was also site specific. At the midcaudal level examined, DA efflux was significantly greater in VTA (1.04 ± 0.05 μM, mean ± SE) than in medial SNc (0.52 ± 0.05 μM), which in turn was higher than in lateral SNc (0.35 ± 0.03 μM). This pattern followed the apparent density of TH-ir, which was also VTA > medial SNc > lateral SNc. This report has introduced a new paradigm for the study of somatodendritic DA release. Voltammetric recording with electrodes of 2–4 μm tip diameter permitted highly localized, direct detection of endogenous DA. The Ca2+-dependence of stimulated release indicated that the process was physiologically relevant. Moreover, the findings that somatodendritic release was frequency dependent across a range characteristic of DA cell firing rates and that stimulated [DA]o varied markedly among DA cell body regions have important implications for how dendritically released DA may function in the physiology and pathophysiology of substantia nigra and VTA.

INTRODUCTION

The electrophysiological and chemical properties of neurons of the substantia nigra pars compacta (SNc) are of interest and importance because of their pivotal role in the pathology of Parkinson’s disease. Nonclassical characteristics such as dendritic release of diverse neurochemicals (Cuello 1982; Geffen et al. 1976; Greenfield 1985; Greenfield et al. 1983) highlight the complexity of these cells. In particular, somatodendritic release of dopamine (DA) from this midbrain cell group (A9) has been a focus of attention since its proposal and confirmation in the mid-1970s ( Björklund and Lindvall 1975; Geffen et al. 1976; Groves et al. 1975; Nieuwenhuijzen et al. 1977).

Most studies of somatodendritic release, including the present one, have focused on the SNc, rather than the adjacent ventral tegmental area (VTA). This is largely because the VTA has DA terminals arising from its own axon collaterals, as well as minor synaptic input from the SNc (Bayer and Pickel 1990; Deutch et al. 1988). Although sparse, the presence of DA terminals can complicate the interpretation of DA release data in this region (Kalivas and Dufy 1991). In contrast, the SNc is devoid of dopaminergic terminals or collaterals (Juraska et al. 1977; Wassef et al. 1981), so that DA release is exclusively somatodendritic in that region.

DA is stored in the dendrites of substantia nigra neurons (Björklund and Lindvall 1975; Cuello and Kelly 1977), although the primary storage site remains controversial. Wilson et al. (1977) first proposed that dendritic storage of DA was in vesicles, as it is in nerve terminals. Subsequent studies, however, suggested that the primary site was in saccules of smooth endoplasmic reticulum (Mercer et al. 1978; Wassef et al. 1981). The issue was readdressed by Groves and Linder (1983), who concluded that storage occurred in both vesicles and endoplasmic reticulum. Consistent with dual storage sites, Pickel’s group recently reported that the vesicular monoamine transporter (VMAT2) is prominently expressed in both of these organelles (Chan et al. 1995).

Speculation about the mechanism of somatodendritic DA release has generated similar controversy. As noted in one of the first reports of somatodendritic release (Geffen et al. 1976), the depolarization and Ca2+-dependence of the release process is consistent with basic ionic requirements for exocytosis. No subsequent reports of the ionic and pharmacological properties of release have disproved this hypothesis (Cheramy et al. 1981; Elverfors and Nissbrandt 1991; Heeringa and Abercrombie 1995; Nieuwenhuijzen et al. 1977; Rice et al. 1994; Santiago and Westerink 1992). On the other hand, the number of vesicles available for exocytotic release is limited (Nireberg et al. 1996; Wilson et al. 1977). Vesicles present appear to be associated primarily with dendrodendritic synapses in the SNc (Groves and Linder 1983; Wilson et al. 1977). Dendrodendritic synapses, however,
are relatively rare in the SNc and absent in the substantia nigra pars reticulata (SNr), so that they comprise <1% of dendritic synapses overall (Groves and Linder 1983). Furthermore, because DA release can be elicited from SNr as well as SNc (e.g., Geffen et al. 1976; Rice et al. 1994), dendrodendritic synapses are not required for release. It is relevant to note, however, that vesicular release of catecholamines can occur in the absence of synapses in other systems, most notably in adrenal chromaffin cells (e.g., Wightman et al. 1991).

Taken together, these data could be interpreted to mean that if vesicles are the primary vehicle for release, the pool of DA available for release is limited, as recently suggested by Heeringa and Abercrombie (1995). Alternatively, the paucity of vesicles as well as DA storage in other organelles have led several investigators to postulate the existence of additional, nonvesicular mechanisms of release (Groves and Linder 1983; Nirenberg et al. 1996; Wilson et al. 1977). There is no experimental evidence as yet, however, to suggest how nonvesicular stores might be linked to a releasable pool.

One feature of somatodendritic release about which there is consensus is that there are few specific synaptic specializations (Cuello 1982; Groves and Linder 1983; Wilson et al. 1977), which suggests that DA relies on extracellular diffusion to reach receptor sites within the substantia nigra (e.g., Groves and Linder 1983). This hypothesis has been strengthened by the recent demonstration that D1 and D2 receptors are largely extrasynaptic in the SNc and the adjacent VTA (Sesack et al. 1994; Yung et al. 1995). These observations support the notion that extrasynaptic communication or “volume transmission” (Fuxe and Agnati 1991; Nicholson and Rice 1991) is important in DA cell body regions. Because the physiological response at DA receptors is concentration dependent (see Lacey 1993), defining factors that regulate stimulated extracellular DA concentration ([DA]o) will be a key step in understanding DA neurotransmission in these regions.

A wide range of techniques has been used to investigate dendritic release of DA from substantia nigra. Release of 3H-DA has been demonstrated in vitro with the use of mesencephalic slices (Chen and Reith 1993; Geffen et al. 1976) and in vivo with push-pull perfusion (Cheramy et al. 1981; Nieoullon et al. 1977). Microdialysis has also been used to monitor extracellular levels of oxogenous and endogenous DA (e.g., Heeringa and Abercrombie 1995; Robertson et al. 1991; Santiago and Westerin 1992). The temporal and spatial resolution of the techniques used, however, have limited the questions that could be addressed with these methods.

We recently reported that voltammetric recording with carbon-fiber microelectrodes and fast-scan cyclic voltammetry (FCV) provides improved resolution for the study of somatodendritic release of DA (Rice et al. 1994). This method permits the detection of submicromolar concentrations of catecholamines on a subsecond time scale (Armstrong-James et al. 1981; Kuhr and Wightman 1986; Rice and Nicholson 1989; Stamford et al. 1986). In our previous study, release of endogenous DA was elicited by veratrine-induced depolarization. A preliminary account of the present studies in which release was elicited with electrical stimulation has been published in abstract form (Cragg et al. 1994).

**METHODS**

**Slice preparation**

Male albino guinea pigs (250–500 g) were anesthetized with halothane and decapitated, and the brain was removed. A block of midbrain or neostriatum was isolated over ice, mounted on the stage of a Vibratome (Lancer Series 1000), and submerged in ice-cold artificial cerebrospinal fluid containing (in mM) 120 NaCl, 5 KCl, 20 NaHCO3, 6.7 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES) acid, 3.3 HEPES salt, 2 CaCl2, 2 MgSO4, and 10 glucose, saturated with 95% O2-5% CO2. Slices 400 μm thick were cut and maintained in individual vials in artificial cerebrospinal fluid for 1 h at room temperature before transfer to the recording chamber at 32°C. The slices were allowed to equilibrate with the superfusion medium for an additional 45 min before the experiment was initiated; the superfusion solution was a modified Ringer solution containing (in mM) 124 NaCl, 3.7 KCl, 26 NaHCO3, 2.4 CaCl2, 1.3 MgSO4, 1.3 KH2PO4, and 10 glucose, saturated with 95% O2-5% CO2. Flow rate was 1.3 ml/min.

The levels of midbrain slices containing the SNc and VTA in this study were generally at A8.0 mm (anterior to the interaural line) in the coordinates of Smits et al. (1990). The presence of the accessory optic tract in these slices was a defining characteristic of this midcaudal level of SNc.

**Carbon-fiber microelectrodes**

Carbon-fiber microelectrodes for use with FCV were prepared from 7- to 8-μm diam carbon fibers (type HM, unsized, Courtreau) in a 2-mm glass capillary tube pulled to a taper. The carbon fiber was spark-etched to extend 30–60 μm beyond the glass insulation, then beveled to a tip diameter of 2–4 μm (Millar 1992). Electrode calibrations were performed in the recording chamber at 32°C in modified Ringer solution containing 1–5 μM DA, 0.5 μM 5-hydroxytryptamine (5-HT), 2–5 μM norepinephrine (NE), or 20 μM 3,4-dihydroxyphenylacetic acid (DOPAC). Calibration solutions were made from stock solutions in 0.1 M HClO4 immediately before use. Electrode sensitivity was determined after use in each brain slice, and individual postslice calibration factors were used to calculate [DA]o. Electrode sensitivity was constant throughout an experimental day, after initial exposure to tissue, and ranged between 1.4 and 13.0 nA/μM. The minimum detection limit for DA was 30–50 nM with the electrodes used in these experiments. The relative sensitivities for DA:5-HT:DOPAC:NE were typically 1:4:<0.015:0.5, respectively.

**FCV**

Voltammetric measurements were made with the use of a Millar Voltameter (P. D. Systems, West Moseley, UK). The instrument was used as a three-electrode potentiostat, with the Ag/AgCl wire as the reference electrode and the stainless steel flow outlet of the bath as the auxiliary. A “microrereference” electrode (Millar and Williams 1990) was not used (Rice et al. 1994).

Voltammograms were obtained in the simple triangle wave mode, with a ramp from −0.7 to +1.3 V then back to −0.7 V versus Ag/AgCl wire (Fig. 1). The dual-waveform setting was employed (Rice et al. 1994), with two identical 5-ms scans separated by 5 ms and repeated at a frequency of 4 Hz. Between scan epochs the electrode was switched out of circuit (Millar and Williams 1990). Scan rate in this mode was 813 V/s. Voltammograms (with background current subtracted electronically) were monitored continuously on a Gould 1602 storage oscilloscope; all experiments...
Chemicals, drugs, and solutions

All drugs and chemicals were obtained from Sigma (Poole, UK). Superfusion solutions were all based on the modified Ringer solution (above). Pargyline was added to the medium for a final concentration of 20 μM; tetrodotoxin (TTX) was added for a concentration of 1 μM; CaCl₂ was omitted in calcium-free (Ca²⁺-free) solutions, but 1 mM ethylene glycol-bis(β-aminoethyl ether)-N,N',N″,N‴-tetraacetic acid (EGTA) and 10 mM MgCl₂ were added; and veratrine (1 mg/ml) was superfused as a 1-ml bolus, with a final chamber concentration of ~0.6 mg/ml.

Histology

Slices were fixed overnight in 4% paraformaldehyde with 75% saturated picric acid in 0.1 M phosphate-buffered saline (pH 7.4) at 4°C. Fixed slices were then resectioned at 50 μm and processed for immunocytochemical localization of TH-ir as described previously (Hajós and Greenfield 1993; Nedergaard and Greenfield 1992).

Experimental design and statistical analysis

All data are expressed as means ± SE. The sample number, n, typically represents the number of slices tested. The substantia nigra is a bilateral structure, so that the [DA], increase at a given site on one side was used as the paired control for release obtained at the corresponding site on the contralateral side under a specific experimental condition (e.g., superfusion of TTX). Data obtained in this way are given as percent of control. Unless otherwise indicated, data points for SNc included both medial and lateral sampling sites. Statistical comparisons were made with the use of paired t-tests or one-way analysis of variance.

R E S U L T S

Confirmation that the released substance was DA

Electrical stimulation (10-s train of 100-μs pulses at 10 Hz) in SNc and VTA in guinea pig mesencephalic slices reliably generated a transient efflux of an electroactive substance detected with the use of carbon-fiber microelectrodes and FCV. This compound, released from dopaminergic regions, gave rise to single oxidation and reduction peaks at roughly 500 and ~200 mV, respectively, versus Ag/AgCl, which were identical to those of DA for a given electrode (Fig. 1b).

The voltammogram of the released substance was readily distinguishable from the voltammogram of 5-HT (Fig. 1c), which exhibits two reduction peaks on the electrodes used (Bull et al. 1990; O'Connor and Kruk 1991; Rice et al. 1994). On the other hand, the voltammogram of DA could not be distinguished from that of its metabolite DOPAC. To confirm the absence of DOPAC contribution to the response, we compared release in control slices with that in slices preincubated for 1–3 h in 20 μM pargyline in HEPES media, then tested in pargyline-containing Ringer solution. Neither the amplitude nor the voltammetric characteristics of the evoked signal differed between control and pargyline-treated slices (P > 0.05; not illustrated). Taken together with the anatomic correlation between release levels and tyrosine hydroxylase (TH-ir), these data confirmed that the released substance was DA.

Characteristics of electrically stimulated DA release from SNc

FREQUENCY DEPENDENCE AND TIME COURSE. Release from SNc was frequency dependent (P < 0.001, F = 5.5) up to 10 Hz, beyond which point no further increase was observed.
FIG. 2. Frequency dependence of electrically stimulated extracellular DA concentration ([DA]₀) in substantia nigra pars compacta (SNc). Bipolar stimulating electrodes were positioned along the compacta band with the tips separated by 100 μm and the voltammetric electrode placed between them. Stimulus parameters were a train of 100-μs, 20-V pulses, delivered for 10 s at frequencies of 1–50 Hz. Release of DA increased with increasing frequency up to 10 Hz, with significant differences between stimulation at 1 or 2 Hz vs. 10 Hz (P < 0.01). Data are means ± SE; n = 4–18 measurements at each frequency.

(Fig. 2). Stimulated increases in [DA]₀ at 10 Hz differed significantly from those at 1 Hz (P < 0.01) or 2 Hz (P < 0.05), whereas there were no differences among higher frequencies. All further characterization of stimulation-evoked DA release was performed with the use of 10-Hz, 10-s pulse trains.

Regardless of frequency, the voltammetric signals obtained during 10-s stimulation trains caused DA release within the first 1–3 s, with a maximum reached by 5–6 s. This maximum was used for calculation of the increase in [DA]₀ at a given site. During stimulation, DA signals were accompanied, then eventually masked, by the progressive distortion of the voltammogram by pH- and possibly Ca²⁺-dependent changes in voltammetric background current (Jones et al. 1994; Rice and Nicholson 1989, 1995; Rice et al. 1994). The confounding influence of these background shifts, which were especially large because of the necessary closeness of the stimulating electrodes to the recording site, precluded quantitative characterization of the kinetics of DA reuptake in this study (e.g., Garris and Wightman 1994; Kawagoe et al. 1992).

Electrical stimulation apparently depleted releasable pools of DA, because no further DA release was detected with a second stimulation given immediately (<1 min) after the initial stimulus. After a 5- to 10-min interval, however, DA release could again be elicited, albeit with lower levels of [DA]₀ than detected initially. Indeed, a second stimulus was never as effective, regardless of the interval between stimulations (5–30 min) or duration of the initial stimulus (1–10 s).

COMPARISON BETWEEN ELECTRICAL AND VERATRINE-EVOKED DA RELEASE. The comparatively high levels of [DA]₀ elicited by veratrine in our previous study (Rice et al. 1994) probably reflected maximal levels of releasable DA in the SNc. In the present study we compared the increase in [DA]₀ evoked by the more discrete electrical stimulation paradigm with that of superfused veratrine. Veratrine induced significantly (P < 0.001) higher [DA]₀ in the SNc than that evoked by 10-Hz, 10-s stimulation (Fig. 3). The mean peak [DA]₀ was 2.45 ± 0.33 (SE) μM (n = 7) with veratrine, which was roughly fivefold greater than that for electrical stimulation, 0.52 ± 0.05 μM (n = 64). Not only was electrical stimulation of SNc a milder stimulus than veratrine, but it was also more selective. Whereas veratrine evoked release of 5-HT, as well as DA, especially in SNr (Rice et al. 1994), the contribution of 5-HT to electrically evoked responses was negligible in either SNc or VTA (e.g., Fig. 1).

COMPARISON BETWEEN DA RELEASE IN STRIATUM AND SNc. Oxidation and reduction peak potentials of DA voltammograms recorded during electrical stimulation (10 Hz, 10 s) of the striatum, which is the major terminal region for SNc cell axons, were identical to those in SNc. Release from striatal DA terminals, however, was significantly higher than from somatodendritic regions of the SNc (P < 0.01). The average increase in [DA]₀ in striatum was 2.78 ± 0.51 μM (n = 10, Fig. 3). In further contrast to release from SNc, increases in [DA]₀ in striatum could be reproducibly elicited with 10-min intervals between stimulations.

TTX AND Ca²⁺ DEPENDENCE OF RELEASE IN SNc. To assess the ionic dependence of electrically stimulated DA release in the SNc, we determined the TTX sensitivity and Ca²⁺ dependence of the release process. Somatodendritic DA release from the SNc in the presence of 1 μM TTX (applied for 30–240 min) did not differ significantly from control release in paired sites (P > 0.05, n = 15) (Fig. 4). DA release from dorsal striatum evoked with the use of the same local stimulation paradigm was similarly TTX insensitive (not illustrated).

By contrast, electrically stimulated DA release in the SNc was markedly Ca²⁺ dependent (Fig. 4). DA release was
Characteristics of Somatodendritic Dopamine Release

Fig. 4. Tetrodotoxin (TTX) and Ca\textsuperscript{2+} dependence of DA release in SNc. Somatodendritic DA release from the SNc was unaffected by the presence of 1 μM TTX (applied for 30–240 min) (P > 0.05, n = 15). By contrast, electrically stimulated DA release in the SNc was Ca\textsuperscript{2+} dependent, with a marked decrease after ≈1 h in Ca\textsuperscript{2+}-free media (**, P < 0.01, n = 15). Data are means ± SE.

decreased to 12 ± 5% of paired controls (P < 0.01, n = 13) after ≈1 h of prior superfusion with Ca\textsuperscript{2+}-free media. Release could be elicited when Ca\textsuperscript{2+} was reintroduced to the media (Fig. 5).

Regional correlation between stimulus-evoked [DA]\textsubscript{o} and TH-ir

To explore regional variation in the release response, we compared stimulated increases in [DA]\textsubscript{o} in medial SNc with those in VTA and lateral SNc (Figs. 6 and 7). These data were further compared with regional variation in the density of subsequent immunocytochemical staining for tyrosine hydroxylase, the enzyme responsible for the final step of DA synthesis (Fig. 7a). Stimulus-induced increases in [DA]\textsubscript{o} were roughly correlated with the intensity of TH-ir across the medial to lateral extent of the A9 and A10 cell body regions of a given slice.

In the slices examined in this report, which were at the level of the mid-caudal SNc, the highest DA levels were obtained in the VTA (1.04 ± 0.19 μM, n = 19), with less in medial SNc (0.52 ± 0.05 μM, n = 64) and least in lateral SNc (0.35 ± 0.03 μM, n = 49) (Fig. 7b). The rise in [DA]\textsubscript{o} in VTA was significantly higher than in medial (P < 0.05) or lateral (P < 0.01) SNc, as was that between medial and lateral SNc (P < 0.01). DA release in SNr rarely reached detectable levels (<30–50 nM), which was consistent with the sparse TH-ir in this area.

Fig. 5. Voltammograms during electrical stimulation of SNc in Ca\textsuperscript{2+}-free and Ca\textsuperscript{2+}-restored physiological saline. Inhibition of DA release in Ca\textsuperscript{2+}-free media was reversed when Ca\textsuperscript{2+} was restored. The [DA], obtained in Ca\textsuperscript{2+}-free media was 0.06 μM, whereas this rose to 0.42 μM in the presence of Ca\textsuperscript{2+}.

Fig. 6. Voltammograms of electrically stimulated [DA], in ventral tegmental area (VTA) and medial and lateral SNc compared with DA calibration voltammogram. a: calibration voltammogram for 1 μM DA (calibration factor in modified Ringer solution at 32°C was 7.6 nA/μM. b: voltammogram of stimulus-evoked [DA], in VTA (0.87 μM). c: stimulus-evoked [DA], in medial SNc (0.53 μM). d: stimulus-evoked [DA], in lateral SNc (0.41 μM). Calibration bar: 3.8 nA. The slice from which these data were recorded was subsequently stained for tyrosine hydroxylase immunoreactivity (TH-ir); the photomicrograph is in Fig. 7a.
One previous report described the coupling of FCV with electrical stimulation in slices of rat midbrain (Stamford et al. 1993). In that study, however, 5-HT was the primary substance detected in the substantia nigra. By contrast, DA was the only substance usually detected in the present studies, which used guinea pig midbrain slices. Preliminary studies comparing stimulated release in rat and guinea pig SNc have confirmed prominent 5-HT release in rat SNc and suggest a species difference in nigral 5-HT innervation between these animals (S. J. Cragg, C. R. Hawkey, and S. A. Greenfield, unpublished data).

The development of a successful protocol to elicit somatodendritic DA release by electrical stimulation represents a marked improvement over veratrine-induced release, as used in our previous study (Rice et al. 1994). The most obvious benefit was that the stimulus was more readily controlled in intensity, duration, and location than was possible with the single, global depolarization induced by superfused veratrine. Unlike electrical stimulation, veratrine elicited 5-HT release in guinea pig SNc 30–50% of the time, depending on recording site (Rice et al. 1994). This suggests contamination of local increase in [DA] with 5-HT released from serotoninergic input to distal dendrites in guinea pig SNr (Nedergaard et al. 1988). The absence of 5-HT detection with electrical stimulation demonstrates the milder, more physiological nature of this stimulus, as well as the more discrete spatial resolution of the experimental paradigm.

**Confirmation of DA detection in SNc**

One limitation of voltammetric techniques is that they do not provide immediate identification of the substance detected. Directly monitored signals obtained with the use of FCV must be validated by supplementary data including in vitro calibration, anatomic verification, and pharmacological manipulation. Several lines of evidence established that we monitored endogenous DA release in the SNc. First, the possibility that the signals might be a nonphysiological stimulation artifact was eliminated because the response was reversibly eliminated in Ca²⁺-free media (Fig. 5). Second, a comparison of calibration voltammograms with the electrochemical signal (Fig. 1) indicated that the released substance was a catechol, like DA or DOPAC, rather than the indole 5-HT. The low sensitivity of the electrodes used for DOPAC (1.0015, DA:DOPAC) suggested that this DA metabolite did not contribute appreciably to the response. This was confirmed experimentally by the lack of significant effect of the monoamine oxidase inhibitor pargyline on amplitude of the release signal in SNc. Another possible interferent, ascorbic acid (ascorbate), was also unlikely to contribute: previous studies indicate that 95% of tissue ascorbate content is lost from guinea pig midbrain in vitro (Rice and Cammack 1991). Even when present, the ascorbate voltammogram is readily distinguished from that of a catechol (Stamford et al. 1984).

Third, the anatomic specificity of the response produced further evidence that the substance monitored was indeed DA. Voltammograms obtained in SNc and VTA were indistinguishable from those obtained in striatum, a region for which DA efflux has been well characterized. Moreover, electrically stimulated increases in purported [DA] could

**DISCUSSION**

In this report we describe a new paradigm for exploration of somatodendritic release of DA in the SNc and VTA. We used carbon-fiber microelectrodes with FCV for direct, in situ detection of DA released by electrical stimulation of DA cell body regions. Voltammetric techniques, especially FCV, have been used extensively to characterize evoked DA release in vivo and in vitro, with most studies focusing on stimulated DA release in striatum (Bull et al. 1990; Garris and Wightman 1994; Gonon and Buda 1986; Kawagoe et al. 1992; May et al. 1988; Millar et al. 1985; Rice et al. 1985; Stamford et al. 1986). The advantages and disadvantages of voltammetric methods have been reviewed elsewhere (Adams 1990; Kruk 1986; Rice and Nicholson 1995).
be obtained only in regions of the substantia nigra and VTA that were positive for TH-ir, with a general correlation between the amplitude of the responses and density of TH-ir at these locations (Fig. 6). Finally, although the voltammetry of DA is also similar to that of the catecholamine NE, additional anatomic and pharmacological studies indicate that NE does not contribute to the release signal in either SNc or VTA (Cragg et al. 1997).

**Frequency dependence of DA release in SNc**

To understand how and where DA can act in DA cell body regions requires not only information about receptor localization (e.g., Sesack et al. 1994; Yung et al. 1995), but also the range of [DA]₀ available to activate these receptors. An important advantage of voltammetric methods is that they permit quantitative evaluation of changes in [DA]₀, that accompany activation of cell populations. In the present studies, stimulus frequencies as low as 1 Hz produced an increase in [DA]₀ of ~200 nM during a 10-s stimulation period. Furthermore, evoked [DA]₀ was frequency dependent between 1 and 10 Hz, then reached a plateau (Fig. 2). This frequency dependence was consistent with the normal range of firing rates of DA neurons: nigral DA cells exhibit two main firing patterns in vivo, single spikes firing at 1–9 Hz (Grace and Bunney 1984a) and bursts of three to eight spikes firing at 6–15 Hz, with interpulse intervals of 200–500 ms (Grace and Bunney 1983, 1984b). The lack of further DA efflux in SNc at stimulus frequencies >10 Hz (Fig. 2) might reflect the limited ability of DA cells to follow frequencies >20 Hz (Grace and Bunney 1983), although it has been reported that frequencies of up to 100 Hz can be followed for short periods (Tepper et al. 1991). Alternatively, the release plateau might reflect depletion of the releasable pool of DA, as discussed further below.

The frequency dependence of DA release in the SNc in vitro was similar to that reported earlier for electrically stimulated DA release from rat striatal slices (Bull et al. 1990). By contrast, both sets of in vitro data differ from the non-linear frequency dependence of [DA]₀, increases in the striatum in vivo after stimulation of the median forebrain bundle (Gonon and Buda 1985; May et al. 1988). These observations may reflect the lower temperature of in vitro studies, which can unmask release processes that are hidden by uptake in vivo. Indeed, Bull et al. (1990) were able to attain detectable levels of [DA]₀ in striatal slices only when the incubation temperature was decreased from 37 to 32°C to decrease DA transporter activity.

**Comparison of somatodendritic and axon terminal DA release**

The most obvious difference in stimulated DA release between somatodendritic and axon terminal regions in the present studies was that increases in [DA]₀ were fivefold lower in SNc than in dorsal striatum with the same stimulation parameters (Fig. 3). Importantly, this difference indicates that the proportion of tissue DA available for release was roughly comparable between these regions, because the DA content of SNc is 12-fold lower than in striatum (Heeringa and Abercrombie 1995). Indeed, at first glance, the data suggest that release was proportionally greater in SNc than in striatum. Other factors, including more avid DA uptake and thus tighter regulation of [DA]₀ in striatum compared with substantia nigra (Cragg et al. 1997; Elverfors and Nissbrandt 1992; Nissbrandt et al. 1991), however, are likely to contribute to these findings.

In SNc and VTA, electrical stimulation evoked the greatest increase in [DA]₀, during the first stimulation at any given site. Although detectable release could again be elicited after several minutes, subsequent stimulations were never as effective. This presumably reflected depletion of releasable DA stores during local stimulation; indeed, evoked [DA]₀ typically reached a maximum during the first 5–6 s of a 10-s stimulus train, then decreased slightly. Depletion of releasable DA also occurs in the striatum after 10-s stimulation of the median forebrain bundle in vivo (Michael et al. 1987). In striatum, however, reproducible release can be elicited repetitively with a sufficient interval between stimulations, both in vivo (Michael et al. 1987) and in vitro (Bull et al. 1990). Greater depletion of releasable DA in the SNc compared with striatum is consistent with previous studies that used amphetamine as a DA releasing agent (Elverfors and Nissbrandt 1992; Heeringa and Abercrombie 1995) and supports the proposal by Heeringa and Abercrombie (1995) that the releasable pool of DA is smaller in somatodendritic than in terminal regions. The concept of the “size” of the releasable pool, however, needs to incorporate implications of proportionally similar DA release in SNc and striatum during an initial stimulus, as discussed above.

Importantly, Bull et al. (1990) reported that when DA uptake was inhibited in striatal slices by GBR 12909, the stimulated increases in [DA]₀ declined rapidly, indicating that active DA uptake is important in maintaining the releasable pool. This suggests that ready depletion of releasable DA stores in SNc and VTA may reflect in part the comparatively lower expression of the DA uptake transporter in SNc and VTA than in striatum (e.g., Ciliax et al. 1995; Cragg et al. 1997; Freed et al. 1995; Kalivas and Duffy 1991).

Taken together, the present findings indicate several differences between the regulation of somatodendritic and terminal release. First, a smaller fraction of somatodendritic DA appears to be stored in a reproducibly accessible pool than that stored in DA terminals. If the primary mode of release were by vesicular exocytosis (Geffen et al. 1976; Heeringa and Abercrombie 1995; Santiago and Westerink 1992), this would be consistent with the limited number of vesicles in DA soma and dendrites of the SNc, compared with those in axon terminals (Groves and Linder 1983; Nirenberg et al. 1996). Second, regardless of release mechanism, the data indicate that replenishment of the releasable pool of DA in the SNc does not occur rapidly, which might reflect limited DA reuptake, as discussed above. Limited uptake would facilitate extracellular diffusion of DA to mediate volume transmission at extrasympathetic DA receptors in the SNc and VTA (Fuxe and Agnati 1991; Greenfield 1985; Groves and Linder 1983; Nicholson and Rice 1991; Yung et al. 1995). This contrasts markedly with the highly organized system of synaptic transmission (Freund et al. 1984; Groves et al. 1994) and avid DA uptake (Kawagoe et al. 1992; Nicholson 1995; Rice and Nicholson 1995) that characterizes axon terminal release in the striatum.
Ionic dependence of DA release

Electrically stimulated DA release in the SNC was Ca\(^{2+}\) dependent (Figs. 5 and 6). The Ca\(^{2+}\) dependence of the process strongly supports the neuronal origin of release, as well as its physiological relevance. Dependence on Ca\(^{2+}\) is also consistent with a key requirement for exocytotic release (Douglas and Rubin 1963; Llinàs 1982). By contrast, stimulated DA efflux was TTX insensitive, even after prolonged exposure to TTX (Fig. 4). Electrically stimulated release of \([^{3}H] DA\) release from midbrain slices reported by Chen and Deutch et al. (1988) was also TTX insensitive. Lack of sensitivity to TTX coupled with the strict Ca\(^{2+}\) dependence of release suggests that local electrical stimulation was sufficient for direct activation of voltage-dependent Ca\(^{2+}\) channels in substantia nigra dendrites (Grace and Onn 1989; Llinàs et al. 1988; Nederhaard and Greenfield 1992). In several previous studies of somatodendritic DA release, sensitivity to TTX was used to address the question of whether somatic Na\(^{+}\) channels, i.e., cell body firing, were required for release. The recent finding that nonattenuating action potentials are mediated by voltage-dependent Na\(^{+}\) channels in DA cell dendrites, as well as in somata (Hàussers et al. 1995), however, limits the adequacy of TTX data to address this issue.

Release of DA in the striatum was also TTX insensitive with the present protocol, which further supports the notion of direct stimulation of Ca\(^{2+}\) entry. This contrasted with a previous study of TTX-sensitive DA release in striatum (Bull et al. 1990), in which the stimulating electrodes were more distant from the recording electrode than in the present protocol, so that DA axons (arising from the median forebrain bundle) were stimulated.

Regional variation in somatodendritic release

The spatial resolution possible with carbon-fiber microelectrodes permitted regional and subregional evaluation of DA efflux in the SNC and VTA. These data provide important complementary information to known differences between these midbrain DA cell populations. The SNC (A9) and the VTA (A10) are the two major subdivisions of the mediolateral continuum of DA cells in the ventral mesencephalon. Although the electrophysiological characteristics of these cells are similar (Lacey 1993), they differ in other key respects. For example, a major projection of SNC neurons is to the dorsal striatum (caudate nucleus), whereas that of VTA cells is to the ventral striatum (nucleus accumbens) (Fallon and Moore 1978). Moreover, mesencephalic DA cells differ in their vulnerability to degeneration in Parkinson's disease: ventral tier cells of the SNC are susceptible, whereas VTA cells are relatively immune (Geman et al. 1988; Gibb and Lees 1991). This has been proposed to reflect biochemical differences between these cell populations, with SNC cells having greater expression of the DA uptake transporter and regulatory D\(_{2}\) autoreceptors, but lower expression of calbindin, a calcium binding protein, compared with cells of the VTA (Blanchard et al. 1994; Ciliax et al. 1995; Freed et al. 1995; Gerfen et al. 1987; Hurd et al. 1994; Sanghera et al. 1994).

The present study demonstrates a further difference between SNC and VTA: DA efflux was twofold to threefold higher in VTA than in SNCs with the same stimulation parameters (Fig. 7b). This was consistent with the threefold higher DA content of VTA compared with SNCs (Rice et al. 1994) and correlated roughly with density of TH-ir across these midbrain regions. Such correlations should be interpreted with caution, however. Although somatodendritic release is characteristic of both VTA and SNCs, release in VTA may also have a component of axon terminal release (see Kalivas and Duffy 1991) from its own axon collaterals, as well as minor synaptic input from the SNCs (Bayer and Pickel 1990; Deutch et al. 1988). Other factors, including greater DA uptake activity in SNCs than VTA (Cragg et al. 1997), will also contribute to differences in [DA]. The methods described in this report are ideally suited for further investigation of dynamic interactions between somatodendritic release and the processes that regulate [DA], in midbrain DA cell body regions.

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