Heterogeneity of Electrically Evoked Dopamine Release and Reuptake in Substantia Nigra, Ventral Tegmental Area, and Striatum

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Cragg, S. J., M. E. Rice, and S. A. Greenfield. Heterogeneity of electrically evoked dopamine release and reuptake in substantia nigra, ventral tegmental area, and striatum. J. Neurophysiol. 77: 863–873, 1997. Somatodendritic dopamine (DA) released in substantia nigra pars compacta (SNc) and the ventral tegmental area (VTA) may mediate extrasynaptic neuronal signaling. The concentration of extracellular DA ([DA]$_{el}$) attained during somatodendritic activation will be governed by the density of release sites and properties of DA uptake. We evaluated these factors in SNc, VTA, and dorsal striatum with carbon-fiber microelectrodes and fast-scan cyclic voltammetry to monitor [DA]$_{el}$, during local electrical stimulation (10 Hz, 5 s) in guinea pig brain slices. Stimulated DA efflux was site specific, with significantly higher [DA]$_{el}$ in caudal (0.48 ± 0.03 μM, mean ± SE) than rostral SNc (0.16 ± 0.01 μM), averaged over their mediolateral extents, and higher [DA]$_{el}$ in VTA (0.74 ± 0.07 μM) than in medial (0.43 ± 0.04 μM) or lateral SNc (0.29 ± 0.05 μM), averaged rostrocaudally. Throughout SNc, evoked [DA]$_{el}$ correlated positively ($r = 0.91$) with the density of tyrosine-hydroxylase-immunoreactive cells. Modulation of evoked [DA]$_{el}$ by uptake was also site specific. The selective DA uptake inhibitor GBR 12909 significantly increased evoked [DA]$_{el}$ in caudal SNc (to 185 ± 27%) and striatum (408 ± 24%), but had no effect in rostral SNc or VTA. Conversely, the norepinephrine (NE) uptake inhibitor desipramine did not alter stimulated [DA]$_{el}$ in caudal SNc or striatum, but caused significant enhancement in rostral SNc (196 ± 17%) and VTA (126 ± 12%). Paroxetine, a selective 5-hydroxytryptamine uptake inhibitor, had little effect in any region tested. Site-specific sensitivity to desipramine mandated evaluation of dopamine-β-hydroxylase immunoreactivity (D/BH-ir) in midbrain. The density of filaments positive for D/BH-ir was greater in rostral SNc and VTA than in caudal SNc, suggesting DA clearance via the NE transporter in these regions. Importantly, D/BH-ir was most dense in sections rostral to SNc where no catecholamine signal was detected and no enhancement was observed with desipramine, indicating a lack of NE contribution to evoked release in any region examined. Taken together, these data confirmed that evoked somatodendritic [DA]$_{el}$ depends on DA cell density and on local uptake properties. Uptake was less efficient in SNc and VTA than in striatum. Moreover, enhancement of stimulated [DA]$_{el}$, by GBR 12909 demonstrated that evoked release from dendrites is not via reversal of the DA transporter. Lastly, the heterogeneous patterns of DA uptake within SNc and VTA were consistent with the pattern of degeneration in Parkinson’s disease: less vulnerable DA cells, e.g., those in VTA, have less DA uptake than the more vulnerable cells of caudal SNc.

INTRODUCTION

Midbrain dopamine (DA) cells comprise a heterogeneous group of cell populations. For example, although resembling a physical continuum, the substantia nigra pars compacta (SNc) (A9) and ventral tegmental area (VTA) (A10) (Dahlström and Fuxe 1964) consist of two functional types of DA cells oriented in tiers: a “dorsal” tier including dorsally situated SNc neurons and cells of the VTA, and a “ventral” tier of ventral SNc neurons (Fallon and Moore 1978; Gerfen et al. 1987b). These midbrain neurons are differentiated in many respects, including cell morphology. Dorsal tier cells of the SNc project dendrites in a mediolateral plane (Fallon et al. 1978), whereas those of the ventral tier also extend dendrites ventrally into the substantia nigra pars reticulata (SNr) ( Björklund and Lindvall 1975; Fallon and Moore 1978). Axons of these cells project differentially as well, with the dorsal tier of SNc projecting to matrix compartments and the ventral tier projecting to patch compartments of the striatum (Gerfen et al. 1987a,b; Jimenez-Castellanos and Graybiel 1987).

These cell types are also discernible biochemically by high (dorsal) and low (ventral) contents of the calcium-binding protein calbindin-D28k (Gerfen et al. 1985). Furthermore, the tiers differ in expression levels of tyrosine hydroxylase (TH), the rate-limiting enzyme of DA synthesis, D$_2$ receptors, and DA transporter genes. Specifically, dorsal-type VTA neurons have lower mRNA and protein levels of both the D$_2$ receptor and the DA transporter than ventral-type SNc cells (Blanchard et al. 1994; Chioido et al. 1984; Ciliax et al. 1995; Freed et al. 1995; Hurd et al. 1994; Sanghera et al. 1994; Shimada et al. 1992).

Importantly, ventral tier SNc cells are more susceptible to degeneration in Parkinson’s disease than dorsal tier cells in either VTA or SNc (Fearley and Lees 1991; Gibb and Lees 1991; Yamada et al. 1990). This pattern of susceptibility is paralleled in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) toxicity (German et al. 1988) and can be prevented by DA uptake inhibition, which implicates DA uptake activity in Parkinsonian degeneration (Javitch et al. 1985; Pfll et al. 1993; Sundström et al. 1986). Determining regional variation in functional DA uptake within the midbrain, therefore, is crucial for understanding both the physiology and pathophysiology of dopaminergic neurons.

A characteristic shared by both dorsal and ventral tier cells is that they release DA from their dendrites (Björklund and Lindvall 1975; Geffen et al. 1976; Groves et al. 1975; Nieoullon et al. 1977). Cells of the SNc, particularly, represent an ideal model population for the study of this release, because, unlike the VTA, the SNc is virtually devoid of dopaminergic axon terminals or collaterals (Juraska et al. 1977; Wassf et al. 1982).

There are also few dendrodendritic synapses in the SNc (Groves and Linder 1983; Wilson et al. 1977), and none in the SNr, so that sites for synaptic DA release in the substantia nigra are limited. Moreover, the DA uptake transporter (DAT) is expressed at sites remote from vesicles in the...
or volume transmission (Fuxe and Agnati 1991; Nicholson dendrites may rely to a large part on extrasynaptic diffusion (Yung et al. 1995). DA receptors are predominantly extrasynaptic in midbrain take in the SNc is underscored by the recent finding that Simon and Ghetti 1993). The relevance of limited DA up-

Taken together, these data suggest that DA released from dendrites may rely to a large part on extrasynaptic diffusion or volume transmission (Fuxe and Agnati 1991; Nicholson and Rice 1991) to its sites of action. Consequently, the time course and specificity of somatodendritic DA signaling may differ from the fast transfer of information characteristic of classical synaptic transmission. Importantly, the sphere of influence of released DA will be regulated by the activity of local DA uptake systems.

In addition to terminating the influence of DA after release, the DAT has also been suggested to mediate somatodendritic DA release by reverse transport (Elverfors et al. 1994; Nirengberg et al. 1996). Although vesicles (for exocytotic DA release) are found in the substantia nigra, they are relatively sparse (Groves and Linder 1983; Nirenberg et al. 1996; Wilson et al. 1977). Moreover, vesicles do not appear to be the primary storage site for dendritic DA, rather DA may be stored in sacules of smooth endoplasmic reticulum (Mercer et al. 1978; Wassef et al. 1982), which can sequester monoamines (Quatacker et al. 1992), possibly via the vesicular monoamine transporter (Nirengberg et al. 1995). Consequently, alternative mechanisms, like reversal of the DAT, have frequently been proposed to take these anatomic data into account (Elverfors et al. 1994; Groves and Linder 1983; Nirengberg et al. 1996). On the other hand, somatodendritic release is strongly Ca

Given that dendritically released DA depends at least in part on volume transmission to its sites of action (Cameron and Williams 1993; Waszczak and Walters 1986; Yung et al. 1995), factors that regulate local [DA]o will determine the efficacy of released transmitter. In the present study we used fast-scan cyclic voltammetry (FCV) with carbon-fiber microelectrodes (Rice et al. 1997) to evaluate factors that regulate [DA]o during electrically stimulated release. These factors included DA cell density, DA cell type, and uptake of DA by dopaminergic and nondopaminergic elements. Regulation of [DA]o by release and uptake in somatodendritic regions was also compared with that in striatum. These data were further used to evaluate the possibility of DAT reversal as a mechanism of somatodendritic release.

**METHODS**

**Brain slice preparation**

Male albino guinea pigs (250–450 g) were anesthetized with halothane and decapitated, and the brains were removed. A block of midbrain, neostriatum, or brain stem was isolated on a glass plate over ice, mounted on a Vibratome (Lancer Series 1000), and bathed liberally in ice-cold artificial cerebrospinal fluid containing (in mM) 120 NaCl, 20 NaHCO3, 10 glucose, 6.7 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES) acid, 5 KCl, 3.3 HEPES salt, 2 CaCl2, and 2 MgSO4, saturated with 95% O2-5% CO2. Slices 400 μm thick were cut and maintained in individual vials in artificial cerebrospinal fluid for ≥1 h before transferal to a recording chamber. The slices were allowed to equilibrate with the superfusion medium at 32°C for an additional 1 h before experimentation. Slices were superfused throughout experimentation at 32°C with modified Ringer solution containing (in mM) 124 NaCl, 26 NaHCO3, 10 glucose, 2.4 CaCl2, 3.7 KCl, 1.3 MgSO4, and 1.3 KH2PO4, saturated with 95% O2-5% CO2. Flow rate was 1.30 ml/min.

The range of levels of midbrain slice used (see Fig. 2) corresponds to coordinates between ~A7.3 and A8.7 mm anterior to the interaural line according to the atlas of Smits et al. (1990).

**Carbon-fiber microelectrodes and FCV**

Carbon-fiber microelectrodes for FCV were prepared from 7- to 8-μm-diam carbon fibers (type HM, unsized, Courtauld) in a 2-mm glass capillary tube pulled to a taper. The carbon fiber was spark-etched to extend 30–50 μm beyond the glass insulation and beveled to a tip diameter of 2–4 μm (Millar 1992).

Electrode calibrations for calculation of extracellular [DA]o were carried out in the recording chamber postexperiment, with 1–5 μM DA, 0.5–2 μM 5-hydroxytryptamine (5-HT), 2–5 μM norepinephrine (NE), or 20 μM 3,4-dihydroxyphenylacetic acid (DOPAC) in the modified Ringer solution at 32°C. Calibration solutions were made immediately before use from stock solutions in 0.1 M HClO4. The detection limit for [DA]o, in situ, was estimated to be 20–30 nM.

Voltammetric measurements were made with the use of a Millar Voltammeter (P.D. Systems, West Moseley, UK). The applied waveform was a triangle wave from ~0.7 to +1.3 V and back to ~0.7 V versus an Ag/AgCl reference electrode (Fig. 1). The stainless steel flow outlet of the recording chamber served as the auxiliary electrode.

Scan rate was 813 V/s and the sampling frequency was 4 Hz.
as described previously (Rice et al. 1997). All current records illustrated are generated by the first of the dual scans, and are faradaic currents obtained by electronic subtraction of the background current. Background-subtracted voltammograms were monitored continuously on a Gould 1602 storage oscilloscope; all experiments and calibrations were recorded on digital audio tape for subsequent analysis.

**Electrical stimulation**

The bipolar stimulating electrodes were prepared from Teflon-coated platinum wire (Clark Electromedical Instruments, Reading, UK), bare diameter 125 μm, coated diameter 175 μm, with a tip separation of 100 μm. With the aid of a binocular microscope, the tips of the bipolar electrode were positioned parallel to the band of cell bodies in the SNc, flush with the tissue for local surface stimulation (Rice et al. 1997). Stimulus parameters were a 5-s (midbrain) or 3-s (striatum) train of 0.1-ms pulses at 20 V and 10 Hz. Maximum evoked [DA], in striatum did not differ between 3- and 5-s stimulations. Stimulus pulses were blanked electronically during voltammetric scanning to prevent interference with the FCV signal. The voltammetric microelectrode was positioned 50–100 μm below the tissue surface, symmetrically between the tips of the stimulating electrode. Correct neuroanatomic positioning of electrodes to regions of the SNc or VTA was enabled by a series of photomicrographs of TH-stained sections.

**Drugs and solutions**

GBR 12909 was obtained from Research Biochemicals International, paroxetine was a gift from Dr. Mihály Hajós, TH and dopamine-β-hydroxylase (DβH) antibody preparations were from Eugene Tech (Ridgefield Park, NJ), and all other chemicals including desipramine were obtained from Sigma (Poole, UK). Fresh 10,000 stock solutions of drugs were made daily in deionized H₂O. All drugs and solutions were applied by superfusion.

**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. Slices were then resected at 50 μm and stained for TH immunoreactivity (TH-ir), as described previously (Hajós and Greenfield 1993; Nedergae and Greenfield 1992), or immunoreactivity to DβH, the enzyme that converts DA to NE, for which the same method was adapted. Slices containing the locus coeruleus were selected as positive controls for DβH immunoreactivity (DβH-ir). Slices processed as above but without the primary antibody served as negative controls. Cell density in SNc was assessed manually at medial and lateral sites with the use of an eyepiece graticule to count TH-immunoreactive cells within a grid of 175 μm (dorsoven- tral orientation) × 350 μm (medial-lateral) through the 50-μm slice depth. Data are given as cells per unit volume, i.e., grid area × 50 μm section thickness. Cell count sites were at midpoints of medial and lateral halves of the SNc. DβH-ir density was determined qualitatively by visual inspection only. Voltammetric data presented with histological results were obtained from the same slices.

**Experimental design and statistical analysis**

Pharmacological studies were conducted by taking control recordings in one hemisphere and experimental condition recordings from paired sites in the contralateral hemisphere. Neuroanatomic landmarks were used to define the DA cell groups: “rostral” SNc (A9) sections were defined as those at the level of the mammillary body and rostral to a supramammillary decussation (A8.7–A8.2); “caudal” sections were caudal to the decussation and mammillary body (<A8.1). Rostrocaudal coordinates were further standardized with reference to the position and size of the accessory optic tract: the tract occupies an identical dorsoventral position to the midbrain DA cell regions and when contained in a given slice is an indicator of the rostrocaudal coordinate, irrespective of plane of cut. SNc (A9) is defined as lateral to the accessory optic tract, and VTA (A10) as medial. All slices were rostral to both the interpeduncular nuclei and any impingement of the medial lemniscus on dorsal SNc.

All data are expressed as means ± SE, and n is number of observations, unless otherwise specified. For the figures, each “mean percent of control” is the mean from a treatment population represented as a percent of the mean of the paired control population. Comparisons for differences in means were assessed by analysis of variance and paired t-tests for means.

**RESULTS**

**Voltammetric characterization of DA signal**

Electrical stimulation of the SNc, VTA, or dorsal striatum in guinea pig slices generated a transient, local faradaic signal (Fig. 1). As reported previously, the evoked signals were frequency and Ca²⁺ dependent (Cragg et al. 1994; Rice et al. 1997). The major oxidation and reduction peak potentials of the voltammogram recorded during stimulation were identical to those of DA (Fig. 1). Other voltammetric and pharmacological studies confirmed further that DA was monitored selectively, rather than interferents including 5-HT or DOPAC (Rice et al. 1997). Basal [DA], was below detection limit.

**Correlation between DA efflux and the density of DA cells in the SNc**

The range of midbrain slices sampled is illustrated in Fig. 2a; the voltammogram obtained in medial SNc in each slice is shown in Fig. 2b. Subsequent to experimentation, slices were fixed and processed for immunoreactivity to TH, the rate-limiting enzyme for DA synthesis. Somatodendritic TH-ir was detected across the entire rostral to caudal range of substantia nigra and VTA preparations from which DA voltammograms could be obtained (Fig. 2b). As suggested previously (Rice et al. 1997), DA efflux was greatest in regions where TH-ir was most dense (Fig. 2).

To investigate the relationship between stimulated [DA], and TH-ir more quantitatively, DA efflux elicited across the rostrocaudal and mediolateral extent of the SNc in a total of 37 slices was compared with the relative number of TH-immunoreactive cells in each area. These comparisons revealed consistent variations in DA efflux that paralleled the density of TH-immunoreactive cells throughout the SNc.

First, DA efflux and cell density showed a marked increase with increasingly posterior coordinate, beginning at ~A8.5 mm (Fig. 3). In general, DA efflux exhibited a rostrocaudal gradient, with an average efflux of 0.03 ± 0.01 (SE) μM (n = 8) at A8.7 mm, increasing in the rostral to caudal direction to 0.57 ± 0.08 μM (n = 36) by A8.0 mm (Fig. 3a). This pattern was mirrored in the relative density of TH-immunoreactive cells in the rostrocaudal plane (Fig. 3b). The small discrepancy in the relationship between [DA], and cell count at the most rostral coordinate of SNc where cell count was zero probably corresponds to DA release...
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FIG. 3. Rostrocaudal variation in DA cell density and stimulated [DA]₀ within SNc. Rostrocaudal coordinates corresponded to those of Smits et al. (1990). a: variation in stimulated [DA]₀ along the rostrocaudal axis of SNc; n = 10–36 measurements from medial and lateral sites combined. b: variation in density of tyrosine-hydroxylase-immunoreactive cells (number of cells per unit sample volume; grid sample volume 175 × 350 × 50 μm) in the same slices as in a (see methods for details); n = 6–22 measurements at each slice coordinate. All data points are means ± SE.

from truncated, isolated DA dendrites that were sometimes observed in this region.

Second, DA efflux and TH-ir density varied across the mediolateral axis of SNc, with greater DA efflux in medial SNc than in lateral SNc, as also noted earlier (Rice et al. 1997). This pattern persisted throughout the rostrocaudal extent sampled in the current study (Fig. 4a): the mean amplitude of DA efflux from medial regions, 0.43 ± 0.04 μM (n = 107), was consistently greater (P < 0.01, F = 8.3) than from lateral regions, 0.29 ± 0.05 μM (n = 74), when compared over the entire rostrocaudal extent of the SNc. A similar mediolateral pattern in the relative density of TH-immunoreactive cells was also seen in the SNc (Fig. 4b, n = 37, P < 0.01, F = 7.5). Indeed, DA efflux throughout the SNc showed a strong positive correlation (r = 0.91) with TH-immunoreactive cell density (Fig. 4c).

The highest evoked [DA]₀ in midbrain regions was seen in VTA (0.74 ± 0.07 μM, n = 29), which is the most medial DA cell body region. Although TH-immunoreactive cells were not counted in VTA, previous studies have shown that DA cell density is higher in VTA than in any part of SNc (German and Manaye 1993). Conversely, slices rostral to the SNc at coordinates more anterior than A8.7–A9.0 mm did not release detectable DA; neither did these areas have somatodendritic TH-ir (see Fig. 2).

Pharmacological characteristics of DA uptake after somatodendritic release

The uptake of DA after stimulated release was examined pharmacologically. GBR 12909 (300 nM), a selective DA uptake inhibitor, significantly enhanced the amplitude of evoked [DA]₀ in caudal SNc to 185 ± 27% of control (n = 21, P < 0.001), corresponding to an evoked [DA]₀ of 0.39 ± 0.10 μM.

FIG. 2. Rostrocaudal range of midbrain regions sampled and corresponding voltammograms obtained in SNc after electrical stimulation. a: range of midbrain regions sampled. Illustrated sections were obtained from different 400-μm slices resectioned to 50 μm and stained for tyrosine hydroxylase immunoreactivity (TH-ir). Coordinates were estimated according to landmarks described in the atlas of Smits et al. (1990). Slices termed “rostal” included those of approximate coordinates A8.7–A8.3 mm, whereas “caudal” were midcaudal slices A8.1–7.5 mm. Scale bar: 500 μm. mb, mammillary bodies; fr, fasciculus retroflexus; aot, accessory optic tract; VTA, ventral tegmental area. b: representative voltammograms obtained during electrical stimulation (10 Hz for 5 s) from medial SNc in slices corresponding to the representative sections in a. Evoked extracellular DA concentration ([DA]₀) ranged from 0.05 to 0.7 μM. Dotted lines: DA peak potentials obtained during postcalibration of the electrode.

FIG. 4. Mediolateral pattern of stimulated [DA]₀ and DA cell density through the rostrocaudal extent of the SNc and correlation between DA efflux and cell density. Rostrocaudal coordinates corresponded to those of Smits et al. (1990). a: variation in stimulated [DA]₀ along rostrocaudal and mediolateral axes of the SNc; n = 5–22 measurements at each slice coordinate. b: density of tyrosine-hydroxylase-immunoreactive cells (number of cells per unit sample volume; grid sample volume 175 × 350 × 50 μm) in the same slices as in a; n = 3–11 slices at each coordinate. c: positive correlation between [DA]₀ and cell density (correlation coefficient r = 0.91). All data points are means ± SE.
of 0.93 ± 0.14 μM (Fig. 5a). Increasing the concentration of GBR 12909 to 3 μM did not cause further enhancement of [DA]₀, (182 ± 24% compared with controls, n = 12, P < 0.01).

By contrast, GBR 12909 had no significant effect on amplitude of rostral SNc signals (112 ± 18%, n = 13, Fig. 5a) at 300 nM, neither at higher (3 μM) nor lower (30 nM) concentrations. Similarly, stimulated release from VTA was not affected significantly by DA uptake inhibition (110 ± 18%, n = 14) (Fig. 5a). In comparison, 300 nM GBR 12909 caused an increase in stimulated efflux from dorsal striatum (caudate-putamen) to 408 ± 24% of control release (n = 6, P < 0.001), equivalent to a maximum [DA]₀ of ~7 μM.

Paroxetine (300 nM), a selective 5-HT uptake inhibitor, did not significantly affect the DA efflux signals compared with controls in either caudal SNc (115 ± 9%, n = 20) or VTA (114 ± 19%, n = 10) (not illustrated). [DA]₀ was marginally, but significantly (P < 0.05), enhanced by paroxetine in rostral SNc compared with paired controls (107 ± 12%, n = 13). Importantly, no significant change in reduction peak potential of the voltammograms was observed (n = 15), which confirmed a lack of 5-HT contribution to the monitored signal, with or without paroxetine.

We further assessed the effect of desipramine, a selective NE uptake inhibitor, on the release response. Stimulated DA efflux from caudal SNc was not significantly different from control with either 50 or 300 nM desipramine (118 ± 12%, n = 8) (Fig. 5b). Furthermore, [DA]₀ in caudate-putamen was not significantly different in the presence of 300 nM desipramine (101 ± 11%, n = 11).

In contrast, evoked [DA]₀ in rostral SNc increased dramatically to 196 ± 17% (n = 11, P < 0.05) (Fig. 5b) in the presence of 300 nM desipramine, equivalent to an increase in [DA]₀ from a control level of 0.15 ± 0.01 μM to 0.30 ± 0.01 μM. Desipramine also caused a significant increase in evoked [DA]₀ in VTA, to 126 ± 12% (n = 15, P < 0.05) of control levels (Fig. 5b), equivalent to an increase in [DA]₀ from a control level of 0.71 ± 0.07 μM to 0.89 ± 0.09 μM.

**Distribution of NE-synthesizing fibers in midbrain**

Although the electrodes used in these studies were twice as sensitive for DA than for the similar catecholamine NE, the peak oxidation and reduction potentials for the two compounds were indistinguishable. To assess whether NE contributed to the release response, especially in rostral SNc and VTA where desipramine enhanced the release response, we mapped the density D/H-ir in midbrain. Brain stem slices containing locus coeruleus showed intense D/H-ir in cell bodies, dendrites, and axons, which served as positive controls for the staining protocol. Ventral midbrain also contained a plexus of D/H-immunoreactive filaments (Fig. 6); in a given coronal slice, D/H-ir was confined to SNc and VTA, with none in SNr, and greater density in VTA than SNc.

Importantly, in the rostrocaudal axis, D/H-ir showed an opposite pattern of density to that of TH-ir: D/H-immunoreactive filaments were more dense in rostral compared with caudal sections (n = 4 sets of serial sections) (Fig. 6), which was also opposite to the pattern of evoked [DA]₀ (see Figs. 2–4). Moreover, midbrain sections that were immediately rostral to TH-immunoreactive soma of the SNc contained the greatest density of D/H-immunoreactive filaments of all midbrain regions examined (apart from locus coeruleus) (Fig. 7a), but did not produce detectable catecholamine release with electrical stimulation (n = 27; Fig. 7b). Furthermore, the lack of a catecholamine response in these rostral sections was unaltered by the presence of 300 nM desipramine (n = 18; not illustrated).

**Discussion**

The results presented here make several important points about somatodendritic DA release and how [DA]₀ is regulated by uptake in SNc and VTA. First, evoked [DA]₀ is proportional to DA cell density throughout the SNc. Second, uptake systems that regulate [DA]₀ after somatodendritic release are less active than those in terminal regions. This disparity suggests a differing form and function of neurotransmission for somatodendritic DA. Third, heterogeneity of active DA uptake systems within the midbrain may further reflect differing roles for dendritic release of DA within discrete subregions. The finding that DA uptake via the DAT is more active in caudal SNc than in the VTA is of considerable importance for understanding the role and sphere of influence of dendritically released DA. For example, it has...
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blockade by desipramine. The absence of catecholamine-like signals in these extreme rostral midbrain sections where DβH-ir was dense suggest that it is highly unlikely that NE contributed to signals recorded in SNc and VTA where DβH-ir was more sparse, either in the presence or absence of desipramine.

Regional variation in evoked [DA]o signal in SNc: correlation with TH-ir

These studies revealed marked regional heterogeneity in evoked [DA]o in the SNc in both rostrocaudal and mediolateral axes. Evoked [DA]o, was greater in caudal than in rostral areas of the SNc. In addition, stimulated [DA]o, was greater in medial compared with lateral SNc, throughout the rostrocaudal axis. These variations in [DA]o, correlated positively with the rostrocaudal and mediolateral density of TH-ir in the SNc (Fig. 4). The data confirm that DA efflux in the SNc was proportional to DA cell density, which in turn suggests a consistent density of release sites per cell.

Regional differences in the effect of DA uptake inhibition

A key requirement of classical neurotransmission is a system for termination of neurotransmitter action following its release; for DA, termination is primarily by reuptake. Previous studies have indicated the presence of the DA transporter in midbrain (Blanchard et al. 1994; Ciliax et al. 1995; Cline et al. 1992; Donnan et al. 1991; Freed et al. 1995; Hurd et al. 1994; Nirenberg et al. 1996; Sanghera et al. 1994; Shimada et al. 1992). Moreover, electrophysiological studies suggest a physiological role for uptake in the modulation of [DA]o in SNc (Lacey et al. 1990; Mercuri et al. 1991; Studer and Schultz 1987), although other studies have questioned the presence of active somatodendritic uptake (尼斯brandt et al. 1991; Simon and Ghetti 1993).

The current report presents evidence for functional DA uptake in DA cell body regions. The selective DA uptake blocker GBR 12909 (300 nM) (Andersen 1989; Bull et al. 1990; Heikkila and Manzino 1984) significantly increased peak evoked [DA]o from SNc in caudal slices, which supports the notion that DA uptake systems play a physiological role in regulating somatodendritic [DA]o in this region. It was not possible to quantify rates of uptake per se in this study, because of the distortion of voltammograms by shifts in pH-dependent background currents following electrical stimulation (Jones et al. 1994; Rice and Nicholson 1989, 1995; see Rice et al. 1997).

The optimal concentration (300 nM) of GBR 12909 for potentiation of stimulated [DA]o, in caudal SNc was the same as that reported previously for striatum (Bull et al. 1990). Importantly, however, the present study reveals that GBR 12909 caused only a twofold increase in stimulated [DA]o, in SNc, whereas the enhancement was fourfold in striatum (Fig. 5a). These data indicate a more avid uptake system in striatum than in caudal SNc (or any somatodendritic region tested) and are in accord with the higher density of catecholamine uptake sites found in nerve terminals compared with cells of origin (Ciliax et al. 1995; Donnan et al. 1991; Freed et al. 1995). An important consequence of less avid uptake in SNc compared with striatum will be a different time

FIG. 6. Photomicrographs of 50-μm sections of SNc stained for dopamine-β-hydroxylase immunoreactivity (DβH-ir). There was DβH-ir throughout guinea pig midbrain, but with rostrocaudal variation: fibers were more dense in (a) rostral SNc (A8.7 mm in the coordinates of Smits et al. 1990) than (b) caudal SNc (A8.0 mm) (n = 4 sets of serial sections). Scale bar: 50 μm.

Does NE contribute to the release signal?

Specific DβH-ir was detected within the SNc and VTA, as reported previously in the guinea pig (Smits et al. 1990) and lizard Gekko gecko (Smeets and Steinbusch 1989). However, the pattern of DβH-ir within the midbrain suggests that if NE were released from DβH-ir fibers, it did not contribute to the voltammetric signal in these regions. The density of DβH-ir was least in caudal and greatest in rostral slices, which is opposite to patterns of both TH-ir and evoked [DA]o. Moreover, DβH-ir was present in regions more rostral to SNc, where no catecholamine signals could be recorded and no enhancement was detected after NE uptake

been estimated that in a DAT-sparse dopaminergic region like medial prefrontal cortex, released DA can travel 6 times farther than DA released in the DAT-dense striatum (Garris and Wightman 1994). On the other hand, uptake of DA into NE elements appears to be especially important in somatodendritic areas like rostral SNc and VTA where DAT activity is weak. Lastly, the data argue against release of DA by reversal of the DAT in SNc or VTA.

Regional differences in the effect of DA uptake inhibition

A key requirement of classical neurotransmission is a system for termination of neurotransmitter action following its release; for DA, termination is primarily by reuptake. Previous studies have indicated the presence of the DA transporter in midbrain (Blanchard et al. 1994; Ciliax et al. 1995; Cline et al. 1992; Donnan et al. 1991; Freed et al. 1995; Hurd et al. 1994; Nirenberg et al. 1996; Sanghera et al. 1994; Shimada et al. 1992). Moreover, electrophysiological studies suggest a physiological role for uptake in the modulation of [DA]o in SNc (Lacey et al. 1990; Mercuri et al. 1991; Studer and Schultz 1987), although other studies have questioned the presence of active somatodendritic uptake (尼斯brandt et al. 1991; Simon and Ghetti 1993).

The current report presents evidence for functional DA uptake in DA cell body regions. The selective DA uptake blocker GBR 12909 (300 nM) (Andersen 1989; Bull et al. 1990; Heikkila and Manzino 1984) significantly increased peak evoked [DA]o from SNc in caudal slices, which supports the notion that DA uptake systems play a physiological role in regulating somatodendritic [DA]o in this region. It was not possible to quantify rates of uptake per se in this study, because of the distortion of voltammograms by shifts in pH-dependent background currents following electrical stimulation (Jones et al. 1994; Rice and Nicholson 1989, 1995; see Rice et al. 1997).

The optimal concentration (300 nM) of GBR 12909 for potentiation of stimulated [DA]o, in caudal SNc was the same as that reported previously for striatum (Bull et al. 1990). Importantly, however, the present study reveals that GBR 12909 caused only a twofold increase in stimulated [DA]o, in SNc, whereas the enhancement was fourfold in striatum (Fig. 5a). These data indicate a more avid uptake system in striatum than in caudal SNc (or any somatodendritic region tested) and are in accord with the higher density of catecholamine uptake sites found in nerve terminals compared with cells of origin (Ciliax et al. 1995; Donnan et al. 1991; Freed et al. 1995). An important consequence of less avid uptake in SNc compared with striatum will be a different time
course and mechanism of action of DA in somatodendritic compared with axon terminal regions, as proposed previously (Greenfield 1985; Groves and Linder 1983; Kalivas and Duffy 1991).

An unexpected finding was the heterogeneity in response to GBR 12909 within SNc itself. In rostral SNc, stimulated [DA]o was not augmented in the presence of GBR 12909 (30–3,000 nM), which would indicate that uptake processes are less active in rostral than caudal regions in clearing [DA]o. Because DA cell density is markedly lower in rostral regions (e.g., Figs. 3 and 4), the simplest explanation may be that the densities of release and uptake sites vary in parallel, with a lower density of somatodendritic DAT and therefore low DA uptake in these regions.

Intriguingly, stimulated [DA]o in VTA was also unaffected by GBR 12909. Unlike rostral SNc, however, the VTA is more cell dense than caudal SNc (German et al. 1993), so that an explanation based on low cell density is not appropriate. These data confirm a mismatch between the density of release and uptake sites in the VTA (e.g., Ciliax et al. 1995), which differs from the relationship in SNc.

The lesser effect of DA uptake inhibition in VTA compared with caudal SNc, therefore, most likely reflects the differing DA cell types in each region. As already described, the VTA contains only dorsal tier neurons with low DAT expression, whereas caudal SNc contains primarily ventral tier cells with higher DAT expression (Hurd et al. 1994; Sanghera et al. 1994). Consequently DA uptake would be expected to be greater in caudal SNc than VTA on the basis of cell type. The present data are the first to demonstrate functional heterogeneity in the regulation of [DA]o by uptake between these regions, as suggested by previous histological studies of DAT expression (Blanchard et al. 1994; Ciliax et al. 1995; Freed et al. 1995; Hurd et al. 1994; Sanghera et al. 1994).

Similarly, it is possible that varying proportions of dorsal and ventral cell types contribute to the heterogeneity of uptake activity within different regions of the SNc. Indeed, Gibb and Lees (1991) described a differential rostrocaudal distribution of dorsal and ventral tier cell populations in human SNc: the dorsal tier extends further rostrally, whereas the ventral tier is dominant in more caudal SNc. Consequently, more dorsal cells in rostral SNc would mean lower DAT levels than in caudal SNc, consistent with our findings (Fig. 5a). Differing rostrocaudal distributions of dorsal and ventral tier cells have not been demonstrated in guinea pig SNc, however, so that rostrocaudal differences in cell density remain the most plausible cause of differences in uptake activity within the guinea pig SNc.

The relative lack of effect of GBR 12909 in VTA in the present studies contrasts with the findings of Kalivas and Duffy (1991), which indicated an increase in basal [DA]o in rat VTA in vivo after DA uptake inhibition by nomifensine. The difference between these results may reflect altered uptake activity during stimulated release compared with basal conditions, as well as other experimental variables. The DAT is Na+/Cl− dependent and electrogenic, so that uptake activity depends on the transmembrane Na∗ gradient and on membrane potential (Kreuger 1990; Wheeler et al. 1993). Consequently, it is likely that DA uptake is inhibited during electrically evoked depolarization. Other contributing factors include the lower activity of DA uptake at 32°C in vitro than at 37°C (Bull et al. 1990) and possible loss of DA from the surface of a superfused slice, which could compete with DAT activity. It should be noted, however, that evoked [DA]o was monitored 50–100 μm below the slice surface, so that the effects of superfusion during a 5-s
sampling period would be minimal (Nicholson and Hounsgaard 1983). In combination, these various factors would be operative in all regions examined, including SNc and striatum. In VTA, however, they are apparently sufficient to mask further DAT inhibition by GBR 12909. Taken with previous results, therefore, the present data should be interpreted to mean less uptake in VTA compared with SNc rather than no uptake.

Evidence against DAT reversal in evoked somatodendritic release

Enhancement rather than diminution of evoked [DA]o in caudal SNc after uptake inhibition argues against reversal of the DAT as a primary mechanism of somatodendritic DA release (Elverfors et al. 1994; Groves and Linder 1983; Nirenberg et al. 1996). This view is further supported by the lack of effect of GBR 12909 in the VTA and rostral SNc. Although the release of DA and other transmitters can be mediated by reverse transport (see Levi and Raiteri 1993), the conditions for the process are usually pharmacological (e.g., by amphetamine or other agents) (Giros et al. 1996; Mercuri et al. 1989; Pifl et al. 1993; Sulzer and Maidment 1993) or pathophysiological (e.g., veratridine, hypoxia, or excitatory amino acid agonists) (Elverfors et al. 1994; Eshleman et al. 1994; Lonart and Zigmond 1991; Sitges et al. 1994). The present data therefore support the physiological nature of electrically stimulated somatodendritic DA release. Moreover, the effects of GBR 12909, together with the strong Ca2+ dependence of the release process (Rice et al. 1997), are inconsistent with release via reversal of the classical DAT, although consistent with characteristics of exocytotic release.

Regional differences in DA uptake by NE fibers

The regional pattern of desipramine influence on stimulated [DA]o, was opposite to that of GBR 12909: desipramine had no significant effect in caudal SNc or striatum, where DAT activity is high, but significantly potentiated [DA]o, in rostral SNc and VTA, where GBR 12909 had no effect. It is unlikely that desipramine acted by inhibiting DA uptake by the DAT, because this action requires concentrations ~100-fold those used in this study (Richfield 1991). The present data therefore suggest that desipramine modulated uptake of DA into NE afferent terminals or fibers via the NE transporter (Carboni et al. 1990; Kelly et al. 1985; Pozzi et al. 1994), although in a regionally dependent manner. Previous studies have indicated that the NE transporter is expressed in low levels in the substantia nigra (Gehlert et al. 1993), whereas evidence from the Weaver mutant mouse suggests that uptake of DA into NE terminals might be a prevalent form of DA uptake in this region (Simon and Ghetti 1993).

Consistent with the greater effect of desipramine in rostral SNc and VTA is our finding that the greatest densities of D/H-ir were found in these regions, with comparatively little D/H-ir in caudal SNc. Indeed, Carboni et al. (1990) have proposed that uptake into NE elements is an important clearance mechanism for extracellular DA in NE-rich areas. Consequently, uptake of DA into NE fibers appears to be an alternative or supplemental mechanism to regulate somatodendritic [DA]o, in regions with weak DA uptake arising from low DA cell density (e.g., rostral SNc) or low DAT expression (e.g., VTA). Greater DA uptake into NE elements in rostral SNc would compensate for lower DAT-mediated uptake and contribute to the linear relationship between cell density and evoked [DA]o, observed throughout SNc (Fig. 4). Conversely, weak DA uptake in rostral SNc and VTA would facilitate uptake of DA into NE terminals, where it could serve as a precursor for NE synthesis by D/H.

Previous studies in the Weaver mouse suggested that DA uptake via the 5-HT transporter is also important in the substantia nigra (Simon and Ghetti 1993). In the present studies in the guinea pig, however, the 5-HT uptake blocker paroxetine (Tulloch et al. 1992) did not significantly enhance stimulated [DA]o, in caudal SNc or VTA and caused only a marginal increase in rostral SNc. Consequently it is unlikely that uptake into serotonergic elements is an important factor in [DA]o, regulation in guinea pig midbrain. As noted previously, however, 5-HT does not contribute to the release signal in guinea pig SNc (Rice et al. 1997) as it does in the rat, so that the effect of 5-HT uptake inhibition might also be species dependent.

Conclusions

The present studies, along with our previous findings (Rice et al. 1997), indicate that [DA]o, in somatodendritic regions is determined by DA cell density, stimulus frequency, and by DA and NE uptake systems. Regional and subregional heterogeneity in these factors leads to locally distinct [DA]o, regulation. A particularly important distinction may be the greater capacity for DA uptake in cells of caudal SNc, which are selectively targeted in Parkinson’s disease, compared with cells of the VTA, which are relatively spared. Demonstration of functional differences in DAT activity between SNc and VTA strengthens the hypothesis that greater activity in cells of caudal SNc contributes to their vulnerability.

The authors thank Dr. Julian Millar for help in the manufacture of the carbon-fiber microelectrodes, Dr. Mihaly Hajós for the paroxetine, L. R. Johnson for advice on histological procedures, and P. Cordery, L. Annetts, S. Deech, C. P. Webb, and M. Preston for technical assistance. This work was funded by Bristol-Myers Squibb. Present address of M. E. Rice: Dept. of Physiology and Neuroscience, NYU Medical Ctr., 550 First Ave., New York, NY 10016. Address for reprint requests: S. J. Cragg, Dept. of Pharmacology, Mansfield Road, Oxford OX1 3QT, UK.

Received 2 February 1996; accepted in final form 9 October 1996.

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