Dopamine-Mediated Volume Transmission in Midbrain Is Regulated by Distinct Extracellular Geometry and Uptake

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Cragg, Stephanie J., Charles Nicholson, June Kume-Kick, Lian Tao, and Margaret E. Rice. Dopamine-mediated volume transmission in midbrain is regulated by distinct extracellular geometry and uptake. J Neurophysiol 85: 1761–1771, 2001. Somatodendritic release of dopamine (DA) in midbrain is, at least in part, nonsynaptic; moreover, midbrain DA receptors are predominantly extrasynaptic. Thus somatodendritic DA mediates volume transmission, with an efficacy regulated by the diffusion and uptake characteristics of the local extracellular microenvironment. Here, we quantitatively evaluated diffusion and uptake in substantia nigra pars compacta (SNc) and reticulata (SNr), ventral tegmental area (VTA), and cerebral cortex in guinea pig brain slices. The geometric parameters that govern diffusion, extracellular volume fraction (\(\alpha\)) and tortuosity (\(\lambda\)), together with linear uptake (\(k\)), were determined for tetramethylammonium (TMA\(^+\)), and for DA, using point-source diffusion combined with ion-selective and carbon-fiber microelectrodes. TMA\(^+\)–diffusion measurements revealed a large \(\alpha\) of 30% in SNc, SNr, and VTA, which was significantly higher than the 22% in cortex. Values for \(\lambda\) and \(k\) for TMA\(^+\) were similar among regions. Point-source DA-diffusion curves fitted theory well with linear uptake, with significantly higher values of \(k\) for DA in SNc and VTA (0.08–0.09 s\(^{-1}\)) than in SNr (0.006 s\(^{-1}\)), where DA processes are sparser. Inhibition of DA uptake by GBR-12909 caused a greater decrease in \(k\) in SNc than in VTA. In addition, DA uptake was slightly decreased by the norepinephrine transport inhibitor, desipramine in both regions, although this was statistically significant only in VTA. We used these data to model the radius of influence of DA in midbrain. Simulated release from a 20-vesicle point source produced DA concentrations sufficient for receptor activation up to 20 \(\mu\)m away with a DA half-life at this distance of several hundred milliseconds. Most importantly, this model showed that diffusion rather than uptake was the most important determinant of DA time course in midbrain, which contrasts strikingly with the striatum where uptake dominates. The issues considered here, while specific for DA in midbrain, illustrate fundamental biophysical properties relevant for all extracellular communication.

INTRODUCTION

When considering how dopamine (DA) mediates volume transmission, the question, “how far does dopamine diffuse?” always arises. As framed, however, the question is incomplete. Without uptake or metabolism to remove DA from the extracellular space, released molecules could diffuse infinitely far. Because extracellular DA concentration ([DA\(_o\)]) will decrease with increasing distance from the source, however, the sphere of DA influence would be limited by the initial number of molecules released and the sensitivity of local DA receptors. Importantly, [DA\(_o\)] would also be influenced by the extracellular volume into which DA is initially released. Moreover, the half-life of [DA\(_o\)] at a given distance from a release site would be governed by the apparent diffusion coefficient of DA. In dopaminergic regions, the sphere of influence of released DA could also be significantly constrained by regionally distinct DA uptake. Here, we determined how these factors regulate DA diffusion in the substantia nigra pars compacta (SNc) and pars reticulata (SNr) and the ventral tegmental area (VTA).

Somatodendritic release of DA in midbrain (Cragg et al. 1997; Geffen et al. 1976; Nieuoullon et al. 1977; Rice et al. 1997) may arise from both synaptic and nonsynaptic sites; DA synapses are few in number in midbrain (Bayer and Pickel 1990; Juraska et al. 1977; Wassef et al. 1981; Wilson et al. 1977), and nonsynaptic release from DA soma has been demonstrated (Jaffe et al. 1998). In addition, DA receptors and the DA transporter (DAT) on DA cell bodies and dendrites are largely extrasynaptic (Niremberg et al. 1996, 1997; Sesack et al. 1994; Yung et al. 1995), as are D\(_1\) receptors on nondopaminergic terminals in these regions (Cameron and Williams 1993; Yung et al. 1995). Thus somatodendritically released DA relies on extracellular diffusion to reach its sites of action, a process known as volume transmission (Fuxe and Agnati 1991; Rice 2000).

The geometric parameters that govern diffusion, outlined above, are the extracellular volume fraction (\(\alpha\)) and the tortuosity (\(\lambda\)) of diffusion paths in tissue (Nicholson and Sykova 1998). In many brain regions, including cerebral cortex and neostriatum, \(\alpha\) is about 0.2, or 20% of brain volume (Csern et al. 1991; Lehmenku¨hler et al. 1993; Pérez-Pinzón et al. 1995; Rice and Nicholson 1991). Tortuosity, \(\lambda\), reflects the increased path-length molecules encounter as they diffuse around cellular elements, compared with that in free solution (Nicholson and Sykova 1998). A consequence of tortuosity is that the apparent diffusion coefficient in tissue, \(D^*\), is decreased from that in solution, \(D\), such that \(D^* = D/\lambda^2\).

The concentration of a diffusing substance will also be
influenced by uptake. For small, nonbiological molecules, like tetramethylammonium (TMA⁺), uptake is low and can be described by the linear uptake term, k' (Nicholson 1992; Nicholson and Phillips 1981). Diffusion of biological molecules, like DA, however, can be profoundly limited by saturable, nonlinear uptake, especially in axon terminal regions like neostriatum (Garris and Wightman 1995; Wightman et al. 1988). Fitting point-source DA diffusion records from striatum to theory therefore necessitates incorporation of Michaelis-Menten uptake parameters (Nicholson 1995; Rice and Nicholson 1995). Somatodendritic uptake of DA in midbrain, however, appears to be less avid than that in striatum (Cragg et al. 1997).

Here, the structural characteristics of midbrain and cortex were defined using the TMA⁺ method of diffusion analysis (Nicholson 1993; Nicholson and Sykóvá 1998) in guinea pig brain slices. Then the unique properties of DA diffusion in midbrain were elucidated using fast-scan cyclic voltammetry (FCV) at carbon-fiber microelectrodes (Rice and Nicholson 1995); specific uptake systems that remove extracellular DA were evaluated pharmacologically. Having established these fundamental characteristics of DA diffusion, we modeled the sphere of influence of somatodendritically released DA.

**METHODS**

**Brain slice preparation**

Male guinea pigs (Hartley strain), 150–250 g, were anesthetized with 40 mg kg⁻¹ ip pentobarbital sodium and decapitated, following National Institutes of Health guidelines and with approval by the New York University School of Medicine Institutional Animal Care and Use Committee. Coronal midbrain slices, 400 μm thick, were prepared as described previously (Rice et al. 1997) using a vibrating-blade microtome (Camden Vibroslice, WPI, Sarasota, FL), with slice coordinates between 7.3 and 8.3 mm anterior to the interaural line (Smits et al. 1990). The presence and location of the accessory optic tract was used to define the appropriate region of midbrain and to delineate SNc (lateral to tract) versus VTA (medial). In some experiments, coronal slices of cerebral cortex (400 μm thick, frontoparietal, motor area) were also prepared. Slices were cut in ice-cold HEPES-buffered physiological saline containing (in mM) 120 NaCl, 20 NaHCO₃, 10 glucose, 6.7 HEPES acid, 5 KCl, 3.3 HEPES sodium salt, 2 CaCl₂, and 2 MgSO₄, saturated with 95% O₂–5% CO₂ for at least 1 h before experimentation (Rice et al. 1994).

Experiments were performed in a submersion recording chamber at 32°C, superfused at 1.3 mL min⁻¹ with bicarbonate-buffered physiological saline, containing (in mM) 124 NaCl, 26 NaHCO₃, 2.4 glucose, 2.4 CaCl₂, 3.7 KCl, 1.3 MgSO₄, and 1.3 KH₂PO₄; saturated with 95% O₂–5% CO₂. For TMA⁺-diffusion measurements, 0.5 mM TMA-Cl was added to the solution. For evaluation of the effect of DA and noradrenaline (NE) transport inhibitors on DA uptake, the selective DAT inhibitor, GBR-12909 (2 mg kg⁻¹), was connected to an EI400 potentiostat (currently available through CyberAmp 320 (Axon Instruments, Foster City, CA). Reference and subtracted ion signals were monitored on a chart recorder and on a digital oscilloscope.

Iontophoresis micropipettes were prepared from theta glass, with tip sizes of 1–3 μm, and contained 150 mM TMA-Cl. Iontophoresis parameters were typically a +100-nA current step on a +20-nA bias, applied using an Axoprobe IA (Axon Instruments, Foster City, CA). The shank of the iontophoresis micropipette was bent to an angle of roughly 30° before filling, so that the shanks of the pipette and the ISM were parallel when the iontophoresis pipette and ISM were glued together with dental cement. Tip separation (r) was 100–160 μm.

Diffusion records for TMA⁺ were first obtained in 0.3% agarose (NuSieve; Rockland, ME) in 150 mM NaCl with 0.5 mM TMA⁺ to obtain the transport number, n, for the iontophoresis pipette in the recording chamber at 32°C. Concentration-time profiles recorded on the digital oscilloscope were transferred to a PC and fitted to the diffusion equation for an iontophoresic point source (Nicholson 1993) using locally written software (VOLTORO and WALTER, both programs written by and available from Nicholson). Values for α, λ, and k' were determined from diffusion curves in brain slices (Nicholson 1993; Nicholson and Sykóvá 1998; Rice and Nicholson 1991) using the following expression

\[ C(r, t) = \frac{Q}{8\pi D^* \alpha r} \left[ h(t, r, \theta) - h(t, r, -\theta) \right] \]

where

\[ h(t, r, \theta) = \frac{g(t, r - d, \theta)}{\sqrt{\pi t}} \exp(-d^2/4D^*t) \]

and

\[ \theta = \sqrt{r^2/2D^*t} \]

In Eq. 1, the concentration at distance r from the source at time t is C; the function H*(r) is the Heaviside step function; the source term Q is the rate of release of TMA⁺ by the source microelectrode. For iontophoresis, Q = IdF, where I is the current and F is Faraday’s electrochemical equivalent; pulse duration is d.

**DA diffusion**

Carbon-fiber microelectrodes (CFMs) were spark-etched to a tip diameter of 2–4 μm and had an active surface extending 30–50 μm below the insulating glass sheath (MPB Electrodes, Queen Mary and Westfield College, London, UK) (see Millar 1992). Electrodes were connected to an EI400 potentiostat (currently available through Cypress Systems, Lawrence, KS). Scan rate for FCV was 900 V/s with a sampling frequency of 4 Hz; scan range was −0.7 to +1.3 V versus Ag/AgCl. The current at the oxidation peak potential for DA (typically +0.6 V vs. Ag/AgCl) was monitored continuously on a chart recorder; DA-diffusion curves were recorded on a digital oscilloscope and transferred to a PC for subsequent analysis using VOLTORO and/or WALTER. Subtraction voltammograms for DA were recorded periodically to confirm signal identity. CFMs were calibrated with 0.5–5 μM DA in bicarbonate-buffered physiological saline in the slice chamber at 32°C; GBR-12909 and desipramine had no effect on
electrode sensitivity for DA at the concentrations of these transport inhibitors used (2 μM). The absolute response time of the electrodes was not determined, however, measurements of DA diffusion in 0.3% agarose with these electrodes yielded the predicted D for DA (not illustrated). That the electrodes could follow the faster rates of change in [DA] in agarose indicates that they should also accurately record diffusion kinetics in tissue.

DA was introduced into the tissue by pressure ejection using a Picospritzer (General Valve, Fairfield, NJ). Pressure ejection, rather than iontophoresis, was used to minimize the duration of DA diffusion curves as well as limit the maximum [DA], in the tissue. Ejection pipettes were prepared from 1 mm OD glass capillary tubing, with tip diameters of 3–5 μm. The shanks of the pipettes were bent; DA backfill concentration (C_b) was 400 μM DA in 150 mM NaCl, with 1 mM thiourea included to prevent DA oxidation. The pipette and the carbon-fiber microelectrode were mounted with shanks parallel and spacing, r, of typically 100 μm on a dual electrode holder attached to a three-dimensional manipulator (HMD-2 and WR-91, Narishige, Tokyo). Pulse pressure and duration were adjusted to give average ejected volumes (U) that produced [DA] at the CFM of approximately 2–3 μM, although a range of [DA], was obtained at each site.

Diffusion of DA was evaluated in SNc, SNr, and VTA. Pressure ejection drop volume for each curve was calculated using the value for α obtained from TMA⁺-diffusion measurements in each region. Concentration-time profiles for DA were fitted using the solution to the diffusion equation for pressure ejection (Nicholson 1985, 1992), again using the program WALTER

\[
C(r, t) = \frac{C_b}{2}\left[\text{erf}(f_+) - \text{erf}(f_-) + \frac{2}{\sqrt{\pi}} \frac{D_c^2}{\alpha^2} \exp(-f_+^2) \exp(-f_-^2)\right] \times \exp(-k't) \tag{2}
\]

where \(f_+ = (r + b)2\sqrt{D^+t}\) and \(f_- = (r - b)2\sqrt{D^-t}\). The variable \(b\) is the initial radius of injected DA, assumed to be deposited instantaneously with a concentration \(C_b\) in the form of a spherical drop. It follows that the volume injected, \(U\), is given by \(U = 4\pi b^3/3\); the amount of substance injected, \(n_{DA}\), is therefore given by \(n_{DA} = UC_b\). Other variables are as previously defined.

As the volume injected becomes small, i.e., \(b \to 0\), and assuming that \(n_{DA}\) remains constant, Eq. 2 tends to the well-known expression for an instantaneous point source (Nicholson 1985, 1992)

\[
C(r, t) = \frac{UC_b}{\alpha(4D^+\pi t)^{3/2}} \exp\left(-\frac{r^2}{4D^+t}\right) \exp(-k't) \tag{3}
\]

Eq. 3 was found to be adequate to describe most of the results and was used for DA diffusion analysis and modeling.

Dextran diffusion

To visualize the ejection of DA and enable further analysis, 1 mM 3000 M_w dextran labeled with the fluorescent probe, Texas Red (Molecular Probes, Eugene, OR) was added to the DA backfill solution, so that DA and dextran co-diffused. Two-dimensional images of dextran fluorescence were obtained using the Integrative Optical Imaging (IOI) method, as described by Nicholson and Tao (1993). The imaging system consisted of a Zeiss Standard microscope equipped with a Nikon ×10 water immersion objective and an epifluorescence illuminator with appropriate excitation and barrier filters for Texas Red. Images were recorded by a charge-coupled device (CCD) camera (Model CH250, Photometrics (now Roper Scientific), Tucson, AZ), cooled to -25°C. The camera was equipped with a Thermo-CCD array with 576 × 384 pixels and 14-bit resolution. Images were transferred directly to a PC for analysis, using software written by Tao and Nicholson. The image intensity along a line through the center of each two-dimensional images, recorded with a 50-ms exposure at 8-10-s intervals, was fitted with a modified form of Eq. 3 that took into account the defocused point spread function of the microscope objective (Nicholson and Tao 1993). This curve fitting permitted the apparent diffusion coefficient of the dextran to be measured, and hence \(\alpha\), but in the present paper, the fitting was simply used to provide qualitative comparisons of ejected volumes under different conditions.

Electrode placement and orientation

Electrode arrays were lowered at an angle of 30° into the slice to a vertical depth of 200 μm from the slice surface. The diffusion path between electrodes was along a dorsal-ventral axis (approximately a 45° angle to the ventromedial-dorsolateral band of cell bodies in the SNc). Exact electrode spacing for each set of diffusion records was determined from images of the electrode array in solution, obtained with the imaging system.

Calculation of DA clearance rates

To estimate clearance rates, the maximum value of \(|dC/dt|\) (in μM s⁻¹) on the falling phase of diffusion curves was determined both for experimental and theoretical records, as described further in RESULTS. The term “clearance” is frequently used to describe removal of DA (or another compound) from the vicinity of a measurement location. The measurement of clearance used here is similar to the chord slope of the change in [DA], between two time points on the falling phase used previously by Gerhardt and co-workers (e.g., Hoffman et al. 1998; Zahniser et al. 1999), but the present measure offered analytical advantages. Both measures of clearance have some limitations, which will be addressed later in the paper. It should be noted, however, that these limitations are relevant primarily for discussions of diffusion from a point source; clearance will have a different significance when other types of release geometry are examined, including stimulated release from a large number of synapses surrounding a measurement site (e.g., Garris and Wightman 1995; Wightman et al. 1988). The present clearance calculations and all analyses using WALTER were carried out with MATLAB (MathWorks, Natick, MA).

Data analysis and statistics

All data are means ± SE; \(n\) is the number of measurements, typically three per region per slice, 2–4 slices per animal. Statistical significance was evaluated using one-way ANOVA, with post hoc, pair-wise Tukey test using SigmaStat (SPSS, Chicago, IL); significance was considered to be \(P < 0.05\).

RESULTS

TMA⁺ diffusion: \(\alpha\), \(\lambda\), and \(k'\) in midbrain and cortex

Geometric factors that govern extracellular diffusion in SNc, SNr, and VTA were evaluated from TMA⁺ diffusion in midbrain slices, monitored using a TMA⁺-ISM; comparative measurements were made in cortical slices. Experimental data were fitted to the diffusion equation for iontophoresis (Eq. 1) to extract \(\alpha\), \(\lambda\), and \(k'\) for each record; the smooth line through each indicates the theoretical curve that fits the data (Fig. 1). In midbrain, \(\alpha\) was generally 0.30 (Fig. 2A), with average values of 0.30 ± 0.01 in SNc (mean ± SE, \(n = 38\)), 0.29 ± 0.01 in SNr (\(n = 34\)), and 0.30 ± 0.01 in VTA (\(n = 26\)). These data contrasted sharply with the \(\alpha\) in cortex, 0.22 ± 0.01 (\(n = 71\)), so that \(\alpha\) was nearly 40% greater in the midbrain (\(P < 0.001\)).

The other geometric parameter, \(\lambda\), was more nearly equal between midbrain and cortex (Fig. 2B). In SNc, \(\lambda = 1.58 ± 0.01\) (\(n = 38\)), in VTA, 1.62 ± 0.02 (\(n = 26\)), and in cortex,
Interestingly, the larger $\lambda$ in SNr compared with cortex caused \([\text{TMA}^+]_o\) in SNr to exceed that in cortex at later time points in these records (Fig. 2D).

$\lambda = 1.59 \pm 0.02$ ($n = 71$). The value of $\lambda$ for SNr, however, was somewhat higher than in SNc, VTA, or cortex ($P < 0.001$), with an average value of $1.69 \pm 0.02$ ($n = 34$).

In contrast to these variations in $\alpha$ and $\lambda$, there were no significant differences in TMA$^+$ uptake among the regions examined, with average $k'$ values of $0.005$–$0.007$ s$^{-1}$ (Fig. 2C).

Theoretical diffusion curves for iontophoretically introduced TMA$^+$ were generated using Eq. 1 with the average values of $\alpha$, $\lambda$, and $k'$ for each region (Fig. 2D). These simulated concentration-time profiles indicate how regional differences in diffusion parameters affected the time course and extracellular concentration of TMA$^+$ ([TMA$^+$_o]). When all other parameters (i.e., diffusion distance, iontophoresis transport number) were held constant, the similar geometric diffusion parameters in SNc and VTA led to nearly identical TMA$^+$-diffusion curves (Fig. 2D). In simulated diffusion curves for SNr, however, [TMA$^+$_o] was initially slightly lower than in SNc and VTA, but exceeded those in SNc and VTA at later times because of the higher $\lambda$ in SNr (Fig. 2B). By contrast, [TMA$^+$_o] in each midbrain region was lower at most times than in cortex, because of the larger $\alpha$ in midbrain (Fig. 2A).
DA diffusion in midbrain: \( \lambda, k', \) and \( U \)

Concentration-time curves for DA after local pressure ejection were recorded in SNc, SNr, and VTA (Fig. 3A). These diffusion records were fitted to theory using the basic diffusion equation for pressure ejection (Eq. 3), with the theoretical fit indicated by the smooth line superimposed on each record (Fig. 3A). The average value of \( \lambda \) determined for each region from TMA\(^+\)-diffusion measurements was used to fit the DA data, so that the ejected drop volume, \( U \), for each record could be calculated. Independent parameters determined in this analysis, therefore were \( \lambda, k', \) and \( U \).

Average values of \( \lambda \) determined for DA diffusion in SNc, SNr, and VTA were slightly (~6%), but significantly, higher than those for TMA\(^+\) diffusion in each midbrain region (\( P < 0.001 \)). Reasons for this are unclear, but it has been suggested that hydroxylated, positively charged amines, including DA, might diffuse more slowly in brain tissue than nonpolar TMA\(^+\) because of cell-surface interactions (Rice et al. 1985). Average \( \lambda \) values were 1.68 ± 0.02 (\( n = 52 \)) in SNc, 1.80 ± 0.02 (\( n = 24 \)) in SNr, and 1.72 ± 0.02 (\( n = 46 \)) in VTA. This pattern of regional differences in \( \lambda \) was similar to that seen with TMA\(^+\): the average value of \( \lambda \) was significantly higher in SNr than in either SNc (\( P < 0.001 \)) or VTA (\( P < 0.05 \)), but did not differ between SNc and VTA.

Diffusion profiles for DA in SNc, SNr, and VTA could be fitted well to Eq. 3 using the linear uptake term, \( k' \) (Fig. 3A). Although linear uptake was also appropriate for fitting TMA\(^+\) curves, the \( k' \) values for DA were over 10-fold larger than for TMA\(^+\) (Fig. 3C; \( P < 0.001 \)), at least in SNc and VTA. In these regions, average DA \( k' \) was 0.085 ± 0.005 s\(^{-1} \) (\( n = 53 \)) in SNc and 0.093 ± 0.006 s\(^{-1} \) (\( n = 45 \)) in VTA (\( P > 0.05 \) for SNc compared with VTA). By contrast, DA diffusion curves in SNr required a \( k' \) of only 0.006 ± 0.001 s\(^{-1} \) (\( n = 24 \)), which was significantly lower than in either SNc or VTA (\( P < 0.001 \)) and indistinguishable from that for nonspecific TMA\(^+\) uptake (Fig. 3C).

Consistent with a linear uptake process, \( k' \) values at a given site were independent of ejection drop volume, \( U \), and hence [DA]\(_o\) over the range examined (Fig. 3B). Indeed, with overall values of \( U \) that ranged from 3 to 150 pL in these experiments, there was no correlation between \( U \) and \( k' \) for DA in any midbrain region (\( R^2 = 0.01 \) (\( n = 53 \)) in SNc; \( R^2 = 0.05 \) (\( n = 24 \)) in SNr; and \( R^2 = 0.22 \) (\( n = 45 \)) in VTA). Maximal [DA]\(_o\) recorded in these regions was on average 2–3 \( \mu \)M, 100 \( \mu \)M away from the point source, although the absolute range was much greater. Conversely, the influence of \( k' \) on \( U \) was indicated by the larger average \( U \) required in SNc and VTA than in SNr to reach similar peak [DA]\(_o\) (Fig. 3A and C). Average values for \( U \) were 41 ± 4 pL (\( n = 52 \)) in SNc; 25 ± 3 pL in SNr (\( n = 24 \)); and 52 ± 8 pL (\( n = 46 \)) in VTA.

DA uptake by DA and NE transporters

To determine the relative contributions of DAT- and NET-mediated processes to uptake of diffusing DA in SNc and VTA, DA diffusion curves were recorded in these regions in the presence of either GBR-12909 or desipramine. Because the \( k' \) for DA in SNc was already similar to that for nonspecific uptake of TMA\(^+\), the inhibitors were not tested in that region. Images of co-diffusing Texas Red–labeled dextran were simultaneously recorded to show the relative size of ejected volumes during the experiment; actual values of \( U \) were calculated from post hoc analysis of DA records, as above. The role of the DAT in regulating [DA]\(_o\) in SNc and VTA was indicated qualitatively by the increased duration of DA diffusion profiles in the presence of GBR-12909 and quantified by the corresponding decrease in \( k' \) (Fig. 4). In the experiment illustrated for SNc...
(Fig. 4A), decreased DA uptake was also indicated by the smaller $U$ required in the presence of the DAT inhibitor to give the same maximum $[\text{DA}]_o$ as in the control record (19 pL compared with 27 pL). The difference in $U$ can also be seen in the companion dextran images (Fig. 4B).

Uptake of DA in VTA was also sensitive to GBR-12909 (Fig. 4C). In the illustrated experiment, $U$ was kept constant between control and uptake-inhibited conditions (31 and 33 pL, respectively), so that the decrease in DA uptake caused an increase in $[\text{DA}]_o$ amplitude throughout the curve in GBR-12909 (Fig. 4C). It should be noted that the falling phases of these records, which reflected the rate of $[\text{DA}]_o$ clearance, were more nearly parallel than those of the normalized records in Fig. 4A, as discussed further below. Images of co-ejected dextran confirmed the similarity of the ejected volumes in these VTA records (Fig. 4D).

**Fig. 4.** Contribution of dopamine uptake transporter (DAT)–mediated uptake to DA diffusion in SNc and VTA. Example DA diffusion profiles (A and C) and simultaneous images of co-diffused Texas Red–labeled dextran (at $t = 20$ s after ejection; B and D) with and without the DAT inhibitor, GBR-12909 (2 $\mu$M). A: in SNc, DA-diffusion records at a given site show decreased clearance rate and lower $k'$ in the presence of GBR-12909; $U$ was adjusted to give similar maximum $[\text{DA}]_o$ with and without GBR-12909 [27 pL in control compared with 19 pL in GBR; $r = 0.99 \mu\text{m}, \alpha = 0.30, \lambda = 1.66$ (control), 1.87 (GBR)]. B: the smaller volume ejected in GBR-12909 was seen in the dextran images in SNc and corresponding Gaussian curve fits of the intensity profile (% of control) along the axis indicated (dotted line); red is high intensity, blue is low. C: in VTA, example DA diffusion profiles show that the decrease in $k'$ in the presence of GBR-12909 increased $[\text{DA}]_o$ throughout the record when similar volumes were ejected [31 pL for control, 33 pL in GBR; $r = 0.99 \mu\text{m}, \alpha = 0.30, \lambda = 1.88$ (control), 1.93 (GBR)]. D: co-ejected dextran images and corresponding Gaussian curve fits for the intensity profile (% of control) along the axis indicated (dotted line) illustrate the similarity of ejected drop volumes.

**Fig. 5.** Comparison of DAT- and norepinephrine uptake transporter (NET)–mediated uptake to DA $k'$ in SNc and VTA. A and B: summary of the effects of uptake inhibition by the (A) dopamine and (B) norepinephrine transporters. A: GBR-12909 (2 $\mu$M) significantly decreased $k'$ from control values in both SNc and VTA ($++P < 0.001$); however, in VTA, $k'$ remained significantly higher than in control SNr (dotted line; $+++P < 0.001$). Data are means ± SE ($n = 17$–53). B: desipramine (2 $\mu$M) decreased $k'$ significantly in VTA ($P < 0.05$), but not SNc. However, $k'$ in desipramine for both SNc and VTA was significantly higher than in control SNr (dotted line; $+++P < 0.001$). Data are means ± SE ($n = 12$–53). C and D: theoretical DA diffusion curves simulated using average parameters to illustrate the effects of DA uptake via the DA- and NE-transporters in SNc (C) and VTA (D). For all simulations: $r = 100 \mu\text{m}; U = 30$ pL; $C_r = 400 \mu\text{M}; D$ for DA at $32^\circ\text{C}$ is $6.90 \times 10^{-6}$ cm$^2$s$^{-1}$; arrow indicates time of ejection.
The overall effect of DAT inhibition on \( k' \) for DA in SNc and VTA is summarized in Fig. 5A. Although GBR-12909 decreased \( k' \) significantly in both regions \((P < 0.001)\), DAT inhibition was more effective in SNc. In the presence of GBR-12909, the DA \( k' \) in SNc decreased to the same level as that seen in SNr \((P > 0.05)\), whereas that in VTA remained significantly higher than in SNr \((P < 0.001)\; \text{Fig. 5A}\). The difference between SNc and VTA was further shown by the significantly lower \( k' \) in SNc compared with that in VTA \((P < 0.05)\) in GBR-12909.

The effect of the NET inhibitor desipramine was less than that of GBR in both SNc and VTA. The slight decrease in the DA \( k' \) in SNc did not reach significance \((\text{Fig. 5B})\). On the other hand, desipramine caused a significant \((P < 0.05)\) decrease in the DA \( k' \) in VTA \((\text{Fig. 5B})\), showing that uptake of \([\text{DA}]_o\) in this region is due in part to the NET, as well as the DAT.

To illustrate the effect of these uptake systems on \([\text{DA}]_o\), simulated DA diffusion curves were generated using a standardized drop volume \((30 \text{ pL})\), average \( \alpha \) and \( \lambda \) values for DA diffusion in SNc \((\text{Fig. 5C})\) and VTA \((\text{Fig. 5D})\), and DA \( k' \) values determined under control conditions or in the presence of GBR-12909 or desipramine. Although the DAT had the greater effect on DA behavior in both regions \((\text{Fig. 5, C and D})\), in VTA especially, NET inhibition also increased amplitude and prolonged the time course of DA concentration-time curves \((\text{Fig. 5D})\).

**Contributions of \( \alpha \), \( k' \), and diffusion to \([\text{DA}]_o\) clearance**

The two most unique characteristics of DA diffusion in midbrain cell body regions were the large \( \alpha \) and the DAT-dependent \( k' \). To understand better the relative contributions of these parameters, we modeled their effects on DA diffusion after pressure ejection in SNc \((\text{Fig. 6})\). Values of \( \alpha \) used were 0.30 \((\text{SNc})\) and 0.22 \((\text{cortex})\), and those for \( k' \) were 0 s\(^{-1} \) \((\text{zero uptake})\), 0.0075 s\(^{-1} \) \((\text{average uptake for DA in SNc in the presence of GBR-12909})\), and 0.085 s\(^{-1} \) \((\text{average control DA uptake in SNc})\).

As predicted from Eq. 3, the larger \( \alpha \) of SNc compared with cortex caused \([\text{DA}]_o\) to be lower at all times, with amplitudes that were proportional to \( 1/\alpha \) \((\text{Fig. 6A})\). These concentration-time curves, in which \( k' = 0 \), also illustrate the role of diffusion alone on clearance of \([\text{DA}]_o\) from a site of a local elevation.

When \( \alpha \) and \( k' \) were varied simultaneously in this model \((\text{Fig. 6B})\), an interesting pattern emerged that showed how these factors, along with diffusion, differentially regulate \([\text{DA}]_o\). Increases in either \( \alpha \) and \( k' \) will cause the amplitude of \([\text{DA}]_o\) to decrease \((\text{Fig. 5, A and B})\) when other parameters \((\text{e.g., amount released})\) are constant. With constant \( \alpha \), the time course of the return to baseline, or clearance rate, of an increase in \([\text{DA}]_o\) will be influenced by \( k' \). For example, when the curve generated for DA diffusion in SNc using \( \alpha = 0.3 \) and \( k' = 0.085 \text{ s}^{-1} \left(\text{Fig. 6B}\right)\) is compared with that generated using \( \alpha = 0.3 \) and \( k' = 0 \left(\text{Fig. 6A}\right)\), an obvious consequence of the higher \( k' \) is that \([\text{DA}]_o\) returned to baseline more rapidly when diffusion alone \((k' = 0)\) was involved. Indeed, when \( k' \) was 0.085 s\(^{-1} \), \([\text{DA}]_o\) decayed to 80% of its maximum by 17 s after ejection \((\text{Fig. 6B})\), whereas when return to baseline involved only diffusion \((k' = 0)\), \([\text{DA}]_o\) had decayed by only 40% at this time and did not fall to 80% until 41 s after ejection \((\text{Fig. 6A})\).

From these observations, the idea easily arises that the rate of fall \((\text{or time derivative of the decay phase})\) following the initial increase in \([\text{DA}]_o\) resulting from a brief, localized release, could be used as a measure of clearance. One can define a maximum clearance rate for \([\text{DA}]_o\) as the absolute value of the minimum \(d[\text{DA}]_o/dt\), at time \( t_{\text{min}} \), of the derivative of the curves in \( A \) and \( B \) for each condition \((d[\text{DA}]_o/dt)\). Both \( \alpha \) and \( k' \) influenced clearance rate.

FIG. 6. Influence of \( \alpha \) and \( k' \) on DA diffusion and clearance. Theoretical DA diffusion curves were simulated using average parameters from SNc. The effect of varying \( \alpha \), with \( k' \) set to 0, is simulated in \( A \); the effects of varying \( \alpha \) and \( k' \) in combination are simulated in \( B \). For all simulations: \( \lambda = 1.58; r = 100 \; \mu \text{m}; \; C_t = 400 \; \mu \text{M}; \) and \( D \) for DA at 32°C is \(6.90 \times 10^{-6} \; \text{cm}^2 \; \text{s}^{-1}\); arrows indicate time of pressure ejection. Values of \( k' \) are those for DA in SNc in the presence \((0.0075 \; \text{s}^{-1})\) and absence \((0.085 \; \text{s}^{-1})\) of GBR-12909. \( C \): maximum DA clearance rate vs. corresponding \([\text{DA}]_o\) plotted for the variations in \( \alpha \) and \( k' \) illustrated in \( A \) and \( B \). The measure of clearance rate was the absolute value of the minimum \(d[\text{DA}]_o/dt\), at time \( t_{\text{min}} \), of the derivative of the curves in \( A \) and \( B \) for each condition \((d[\text{DA}]_o/dt)\). Both \( \alpha \) and \( k' \) influenced clearance rate.

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Sphere of influence of DA after local release in SNc and SNr

Knowing the geometric constraints on DA diffusion and the regionally distinct characteristics of DA uptake in midbrain, we could then address how these parameters might influence [DA]o following local release in SNc and SNr. Because diffusion parameters determined here for SNc and VTA were similar, calculations for SNc would also apply to VTA. Average α, λ, and k’ for SNc and SNr were used in Eq. 3 to generate theoretical curves. The point source was considered to be a site of DA vesicle fusion on a DA cell body or dendrite. Based on an estimated 14,000 DA molecules per vesicle in a SNc neuron, as previously reported by Jaffe et al. (1998), if 20 vesicles were released, the amount of DA entering the extracellular volume would be 280,000 molecules, or 4.6 × 10⁻¹⁹ moles. The simulation further used an intravesicular [DA] of 350 mM (Cv) in a vesicle volume of 6.5 × 10⁻²⁰ L (U), calculated from a vesicle radius of 25 nm (e.g., Nirenberg et al. 1997). The diffusion distance selected for these calculations was 20 μm, reflecting a typical distance between DA dendrites in SNr (Fig. 7). The maximum [DA]o after “release” under these conditions was 13.7 nM in SNc at t max = 259 ms and 14.4 nM in SNr at t max = 276 ms (Fig. 7). These concentrations were comparable to known EC 50 values for D₂ receptor activation (1–20 nM) (Levant 1997). Maximum clearance rate, d[DA]o/dt, in SNc was 0.020 μM s⁻¹ and in SNr was 0.016 μM s⁻¹. The difference in clearance rate between SNc and SNr also influenced clearance times after the [DA]o peak: the time required for the [DA]o maximum to decay by 50% (t 1/2) was 490 ms in SNc and 580 ms in SNr, with 1.29 s required for 80% decay in SNc and 1.60 s in SNr.

Discussion

After somatodendritic release in midbrain, DA acts at extrasynaptic receptors (Sesack et al. 1994; Yung et al. 1995) via diffusion-based volume transmission. We describe here the factors that govern the lifetime and concentration of DA in SNc, SNr, and VTA. Both geometric parameters and specific DA uptake were quantified. Together, these data provided a complete picture of DA regulation in midbrain that permitted us to model the behavior of [DA]o after simulated vesicular release.

TMA⁺ diffusion

From TMA⁺ diffusion data, the extracellular volume fraction of midbrain was shown to be 0.30. This value of α was 40–50% larger than the usual 0.18–0.22 found in cerebral cortex, both in the present studies in guinea pig and in previous studies in rat (Cserz et al. 1991; Lehmenkühler et al. 1993; Pérez-Pinzón et al. 1995). Moreover, α is also about 0.2 in neostriatum, cerebellum, hippocampus, and spinal cord across species (Nicholson and Phillips 1981; Pérez-Pinzón et al. 1995; Rice and Nicholson 1991; Rice et al. 1993; Svoboda and Sykova 1991). Subregional values can differ from 0.2, however, with an α of about 0.3 in the cerebellar molecular layer (Rice et al. 1993) and in the hippocampal stratum pyramidale (Mazel et al. 1998). Even higher values of α occur in immature rat cortex, where 40% of cortical volume is extracellular during the first days of postnatal development (Lehmenkühler et al. 1993). At that stage, neurons are not yet ensheathed in the proteoglycan complexes that comprise perineuronal nets (Celio and Blumcke 1984). A contributing factor to the large α of midbrain therefore might be that catecholamine neurons, including midbrain DA cells, do not have perineuronal nets, even at maturity (Hobohn et al. 1998).

The strikingly higher value of α in midbrain will cause DA behavior to differ significantly from that in forebrain. Whenever a given number of molecules enter the larger extracellular space of midbrain after release from either an exogenous or endogenous source, the resultant [DA]o will be lower than in forebrain. This has obvious implications for experimental comparisons of [DA]o between midbrain and forebrain structures. More importantly, this means that concentration-dependent receptor activation will be lower in midbrain after equivalent DA release.

Despite the large α of midbrain regions, the corresponding tortuosity factors were similar to that of cortex. This relative constancy in λ might seem surprising; however, it should be noted that α and λ are mathematically independent parameters (Nicholson and Phillips 1981). A similar invariance in λ is also seen in developing rat cortex, in which λ is constant as cortex matures from the neonatal period to adulthood, while α decreases from 0.4 to 0.2 (Lehmenkühler et al. 1993). Within midbrain, λ was significantly higher in SNr than in SNc or VTA. Although the difference was <7%, this had subtle effects on DA time course (Fig. 2).

DA diffusion in midbrain

DA-diffusion curves in midbrain could be fitted well to the diffusion equation (Eq. 3) using linear uptake, k’, and, thus did not require more elaborate fitting procedures incorporating nonlinear Michaelis-Menten uptake kinetics (e.g., Nicholson 1995). By contrast, similar DA-diffusion data from striatum cannot be fitted with k’, and require Michaelis-Menten kinetic parameters (Rice and Nicholson 1995). The suitability of linear uptake to describe the present data would suggest that the concentrations examined (low micromolar) were within a
range in which the relationship between uptake rate and concentration was approximately linear. One condition under which the relationship between Michaelis-Menten uptake rate and concentration is linear is when $[\text{DA}]_o$ is substantially below $K_m$ (i.e., $C \ll K_m$), such that the nonlinear uptake term $V_{\text{max}}C/(C + K_m)$ simplifies to $(V_{\text{max}}/K_m)C$ (i.e., $k' = V_{\text{max}}/K_m$).

In the present experiments, this would mean that $K_m$ was $\gg 2\sim 3 \text{ } \mu\text{M}$, which was our average peak $[\text{DA}]_o$. A $K_m$ of this magnitude seems unlikely, however, given that the usual DAT $K_m$ is about 0.2 $\mu\text{M}$ [although values $> 1 \mu\text{M}$ have also been reported (Giros and Caron 1993; Horn 1990)]. On the other hand, it is relevant to note that Michaelis-Menten kinetics describe initial uptake rates, a condition that may not be met in many experimental paradigms nor during normal release in situ.

The appropriateness of using linear uptake to fit DA diffusion curves in the present studies in midbrain was confirmed by the independence of $k'$ from $U$, such that similar $k'$ values were obtained in each of a family of DA curves of different amplitude (Fig. 3B). Moreover, the physiological relevance of $k'$ was indicated by its sensitivity to transporter inhibition (Figs. 4 and 5). Experiments with DAT and NET inhibitors indicated that most DA uptake in SNc and VTA was via the DAT. This is consistent with the high density of DA cell bodies and dendrites expressing the DAT in these areas (Ciliax et al. 1995; Freed et al. 1995; Nirenberg et al. 1996, 1997). Previously, we reported that DAT inhibition enhanced maximum evoked $[\text{DA}]_o$ in SNc, but had no effect in VTA (Cragg et al. 1997). The present studies showed that active DA uptake does occur in VTA, with control values of $k'$ that were similar to those in SNc. Nonetheless, here, too, DAT inhibition was more effective in SNc than in VTA, demonstrating a functional consequence of the lower density of DAT expression in VTA (e.g., Ciliax et al. 1995). In our previous study (Cragg et al. 1997), the depolarizing stimulus used to evoke DA release was presumably sufficient to inhibit the voltage-sensitive DAT (Hoffman et al. 1999), thus masking additional inhibitory effects of GBR-12909. This was more evident in VTA, where DAT activity is lower, than in SNc. In contrast to DAT inhibition, inhibition of the NET only slightly decreased DA $k'$ in VTA and SNc, reaching significance only in VTA. Uptake of DA by the NET (Cragg et al. 1997; Simon and Ghetti 1993) in VTA appears to be by en passant NE processes (Cragg et al. 1997).

In contrast to SNc and VTA, in SNr $k'$ for DA was indistinguishable from that for TMA$^+$. Although the DAT is expressed on DA dendrites in SNr, as in SNc, the distribution of TH-ir-positive processes is sparser in SNr (Fig. 7). Consequently, DA can diffuse for relatively large distances in SNr without encountering uptake sites, as indicated by our experimental results (Fig. 3, A and C). This geometric organization is somewhat analogous to that in retina, where DA is released from dopaminergic amacrine cells that are oriented in a plane defined by the inner plexiform layer. Once DA escapes these cells, it is free to diffuse to DA receptors on horizontal cells and photoreceptors 50–100 $\mu\text{m}$ away (Rice 2000; Witkovsky et al. 1993).

Relevance for DA volume transmission

The effects of $\alpha$ and $k'$ on DA diffusion in SNc were modeled to evaluate how these parameters influence $[\text{DA}]_o$ and DA clearance rate. For these comparisons, clearance was taken as the maximum $[\text{d}[\text{DA}]_o/\text{d}t]$ of the falling phase of a diffusion curve. A previous study (Hoffman et al. 1998) also examined DA clearance following pressure ejection and found surprisingly similar values for clearance rate, despite a threefold greater diffusion distance. In those experiments, DA uptake inhibitors caused an increase in $[\text{DA}]_o$ amplitude and time course, but paradoxically did not alter clearance rate (Hoffman et al. 1998). The present studies, in which diffusion parameters and DA uptake were evaluated independently, resolve this paradox. Here we show a clear decrease in DA $k'$ in the presence of a DAT inhibitor; we also show that the concentration dependence of DA clearance rate precludes its use for adequate assessment of uptake blockade. Moreover, our models of DA diffusion indicated that for a given amount of “released” DA, a region with a larger $\alpha$ or $k'$ would have lower $[\text{DA}]_o$. Although a large $k'$ increased $[\text{d}[\text{DA}]_o/\text{d}t]$, a large $\alpha$, with constant $k'$, decreased it because of the lower $[\text{DA}]_o$. A large $k'$ also decreased clearance time, whereas different $\alpha$ values did not affect this. The overall effect of the large $\alpha$ of midbrain, therefore would be to decrease $[\text{d}[\text{DA}]_o/\text{d}t]$, effectively damping changes in $[\text{DA}]_o$.

When all now-known diffusion characteristics were combined with specific DA uptake, we could then model DA diffusion in SNc and SNr following vesicular release (Fig. 7). For this model, we assumed a 20-vesicle point source, with 14,000 DA molecules per vesicle (Jaffe et al. 1998). This gave a peak $[\text{DA}]_o$ of 14 $\text{nM}$ that occurred roughly 250 $\text{ms}$ after release, 20 $\mu\text{m}$ away, whether in SNc or SNr. This concentration is similar to affinity constants for the high-affinity state of all DA receptor subtypes (Neve and Neve 1997) and to EC$^{50}$ values for D$_1$-like (Jackson et al. 2000) and D$_2$-like (Levant 1997) receptor activation in vitro. Consequently, 14 $\text{nM}$ could be sufficient to activate most high-affinity receptors within this radius. Equally important, in both regions, these concentrations would be available for a sufficient time for DA receptor activation (Cragg and Greenfield 1997): $t_{1/2}$ was nearly 500 $\text{ms}$ in SNc and 600 $\text{ms}$ in SNr.

The most significant result of this modeling, however, was the surprising similarity of the $t_{1/2}$ values in SNc and SNr, despite the 10-fold difference in $k'$ in these structures. This shows, for the first time, that diffusion rather than uptake is the most important determinant of DA time course in midbrain. The dominance of diffusion contrasts completely with the case in striatum in which uptake dominates (Garris and Wightman 1995; Giros et al. 1996).

Conclusions

The large $\alpha$ and limited, linear uptake of DA in midbrain will act as “oscillation dampers” to decrease the amplitude and rate of fluctuation of $[\text{DA}]_o$ to which extracellular DA receptors are exposed. These findings not only allow us to predict the behavior of somatodendritically released DA, but also have implications for all biogenic amine systems in midbrain. Indeed, these novel results illuminate concepts that are relevant for any substance that mediates volume transmission.

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