Exp Brain Res (1994) 100:395–406

ORIGINAL PAPER

M. E. Rice · C. D. Richards · S. Nedergaard
J. Hounsgaard · C. Nicholson · S. A. Greenfield

Direct monitoring of dopamine and 5-HT release in substantia nigra and ventral tegmental area in vitro

Received: 7 September 1993 / Accepted: 11 March 1994

Abstract Fast-scan cyclic voltammetry with carbon fibre microelectrodes was used to detect endogenous dopamine (DA) and 5-hydroxytryptamine (5-HT) release from three distinct regions of guinea-pig mid-brain in vitro: rostral and caudal substantia nigra (SN) and the ventral tegmental area (VTA). Previous electrophysiological studies have demonstrated that cells of the caudal SN and the VTA have similar characteristics, whereas cells in the rostral SN have distinctly different properties. In the present study, we confirmed that each region has tyrosine hydroxylase-positive neurons and determined, using high-performance liquid chromatography, that DA levels were similar in rostral and caudal SN, but lower in SN than in VTA. In each region, application of veratrine, which was shown by intracellular recordings to have a reversible depolarising action, evoked a signal attributable to DA and distinguishable from that of 5-HT. Release signals were monitored every 250 ms with a spatial resolution of less than 50 μm. DA release was calcium-dependent and was not detectable in a catecholamine-poor area such as the cerebellum, or in mid-brain tissue pre-treated with reserpine. Within the normal mid-brain, the amount of DA released was correlated with tissue content in that it was higher in the VTA than in either region of SN. It is concluded that DA released from somato-dendritic parts of mid-brain neurons exhibits site-specific variation. This is the first report of direct monitoring of DA and 5-HT relase from these regions with in situ electrodes and demonstrates the utility of fast-scan cyclic voltammetry to investigate the mechanisms and possible non-classical functions of somato-dendritic DA release.

Key words Dopamine release · Fast-scan cyclic voltammetry · Mid-brain · 5-Hydroxytryptamine Guinea-pig

M. E. Rice · C. Nicholson
Department of Physiology and Biophysics,
NYU Medical Center, 550 First Ave., New York,
NY 10016, USA

C. D. Richards (✉) · S. A. Greenfield
University Department of Pharmacology, Mansfield Road,
Oxford OX1, 3QT, UK, Fax no: +44-865-271853

S. Nedergaard
Institute of Physiology, University of Aarhus,
DK-8000 Aarhus C, Denmark

J. Hounsgaard
Panum Institute, University of Copenhagen, Blegdamsvej,
DK-2200 Copenhagen N, Denmark

Introduction

Mid-brain dopamine (DA) neurons have been the focus of much attention because of their pivotal role in the pathology of Parkinson's disease. It is of particular interest therefore that these neurons appear to display novel, non-classical characteristics, including the dendritic release of DA (Cheramy et al. 1981; Geffen et al. 1976; Kalivas and Duffy 1991; Tagerud and Cuello 1979) and other neurochemicals (Greenfield et al. 1983). Within the substantia nigra (SN) there are virtually no dopaminergic (or indeed any catecholaminergic) axon terminals or collaterals (Cuello and Kelly 1977; Juraska et al. 1977; Parizek et al. 1971; Sotelo 1971). Local DA release from somato-dendritic parts of the neuron differs from that at the striatal nerve terminal in several critical respects. First, the transmitter originates not from vesicles but from smooth endoplasmic reticulum (Mercer et al. 1978; Wassef et al. 1981); secondly release is unrelated to cell excitability (see Greenfield 1985) and is unaffected by tetrodotoxin (TTX) in certain conditions (Cheramy et al. 1981), although not in others (Santiago and Westerink 1990; Santiago et al. 1992); thirdly, although specific appositions have been reported in the pars compacta (Wilson et al. 1977), there are no specific target sites in direct apposition to the release sites, i.e. no dendro-dendritic synapses within the pars reticulata (Cuello 1982; Wassef et al. 1981). It is not surprising, therefore, that dendritic release of DA has been proposed to underscore a form of neuronal signalling alto-

gether distinct from that at the classic axonal synapse (Cheramy et al. 1981; Greenfield 1985; Groves et al. 1975).

The physiology of dendritic DA release becomes even more intriguing in the light of relatively recent evidence suggesting a possible heterogeneity of DA neurons within the mid-brain. Within the SN itself there appear to be two distinct sub-populations of presumed dopaminergic cells, in the rostral and in the caudal pars compacta (Chiodo et al. 1980; Nedergaard and Greenfield 1992), which display distinct patterns of discharge, 'phasic' and 'rhythmic' respectively, in vitro (Nedergaard and Greenfield 1992). It has been proposed that the low-threshold calcium spike associated with phasic cells may play a critical role in the responsiveness of dendrites to synaptic input (Llinás et al. 1984). Consequently, dendritically released DA might have differential release characteristics and effects in each of these mid-brain DA regions.

It has been impossible to date to explore dendritic release of DA with a time-space resolution commensurate with physiological events. The sensitivity of existing assays coupled with microdialysis or push-pull perfusion, whether through the conversion of radiolabelled precursor (Nieoullon et al. 1977) or detection of endogenous transmitter using high-performance liquid chromatography (HPLC) (Westerink et al. 1987), has restricted the speed of monitoring to periods of 10 and 15 min respectively. This long sampling interval, when combined with the spatial extent of local perfusion devices such as push-pull cannulae (Cheramy et al. 1981) or microdialysis assemblies (Robertson et al. 1991), has limited the spatial resolution of previous studies to over a millimetre. Other methods using whole-tissue release from brain slices (Elverfors et al. 1992; Geffen et al. 1976; Tagerud and Cuello 1979) have even lower spatial resolution.

The spatial and temporal limitations of previous sampling methods can be circumvented by using fast-scan cyclic voltammetry (FCV) with 8 μm diameter carbon fibre microelectrodes, which can directly monitor concentration changes in extracellular DA at a release site on a sub-second time scale (Kuhr and Wightman 1986; Rice and Nicholson 1989; Stamford et al. 1986). FCV has been developed extensively over the last 10 years (Armstrong-James et al. 1981; Millar et al. 1985) to monitor, most usually in the striatum, the extracellular concentration of either endogenous (Bull et al. 1990; Millar et al. 1985) or iontophoretically introduced DA (Armstrong-James et al. 1981; Rice and Nicholson 1989; see also Nicholson and Rice 1988 and Adams 1990). These approaches have not as yet been directed towards exploring dendritic release of endogenous DA within the SN.

In this study we used FCV to monitor evoked release of endogenous DA in three distinct regions of mid-brain: rostral and caudal SN, and the ventral tegmental area (VTA), where dendritic release is also known to occur (Kalivas and Duffy 1991). DA release signals were correlated with regional cell electroresponsiveness, tyrosine hydroxylase staining, and DA content. Veratrine was chosen as an appropriate secretagogue, since it has been long established as a potent agent for evoking release of DA within the SN (Tagerud and Cuello 1979), and since its molecular properties ensured minimal interference with the FCV measurement. In a separate series of experiments, the effects of veratrine treatment were also observed on the membrane properties of individual mid-brain neurons using intracellular recording. Veratrine-induced depolarisation was also expected to release 5-hydroxytryptamine (5-HT), as predicted by earlier reports (Hery et al. 1980; Reubi and Emson 1978); however, contributions from DA and 5-HT could be distinguished on the basis of their characteristic voltammograms. In order to confirm that the voltammetric signal corresponded to released biogenic amines and not to an artefact of stimulation, FCV measurements were made in tissues with little endogenous DA, namely the cerebellum and regions of mid-brain from animals pre-treated with the catecholamine-depleting agent, reserpine.

A preliminary account of this study has been published elsewhere (Richards et al. 1993).

Materials and methods

Slice preparation

Male albino guinea-pigs (300–500 g) were anaesthetised with halothane, decapitated and the brain removed. A block of mid-brain, forebrain or cerebellum was isolated and mounted on a Vibratome (Lancer Series 1000) and bathed in cold artificial cerebrospinal fluid (HEPES-acsf), which contained (in mM): NaCl 120, KCl 5, $NaHCO_3$ 20, $MgSO_4$ 2, KH_2PO_4 1.25, $CaCl_2$ 2, HEPES acid 6.7, HEPES salt 3.3, glucose 10; gassed with 95% O_2/5% CO_2 (modified from Llinás and Sugimori 1980). From these blocks, 400 μm thick slices were cut containing the SN and/or VTA, striatum, or molecular layer of the cerebellum. The levels of mid-brain slice containing the SN and VTA are shown in Fig. 1. These sections correspond roughly to 7.5 mm in front of the interaural line for rostral slices and 6.5 mm for caudal, using coordinates for a 400 g guinea-pig (Luparello 1967). Note the characteristic features at each level: in rostral sections, mamillary bodies are conspicuous, while the accessory optic tract is discernible in more caudal sections.

Slices were maintained in individual vials in HEPES-acsf for at least 1 h prior to use, then transferred to a recording chamber and superfused with modified Ringer's solution at 32° C, which contained (in mM): NaCl 124; KCl 2; $NaHCO_3$ 26; $MgSO_4$ 1.3; KH_2PO_4 1.25; $CaCl_2$ 2.4; glucose 10; gassed with 95% O_2/5% CO_2 (modified from Llinás and Sugimori 1980). Veratrine was prepared at a concentration of 1 mg/ml, and a 40 s pulse of this solution was applied (flow rate 1.5 ml/min), resulting in an approximate final bath concentration of 0.6 mg/ml.

Intracellular recording

Recordings were made as previously described (Llinás et al. 1984; Nedergaard and Greenfield 1992). Glass microelectrodes filled with 3 M potassium acetate (series resistances measured in Ringer's solution: 50–100 MΩ) were mounted on a micromanipulator and positioned, with the aid of a dissecting microscope, in either the SN pars compacta or the VTA. Intracellular potentials were passed into a Neurodata (IR-283) preamplifier with an active

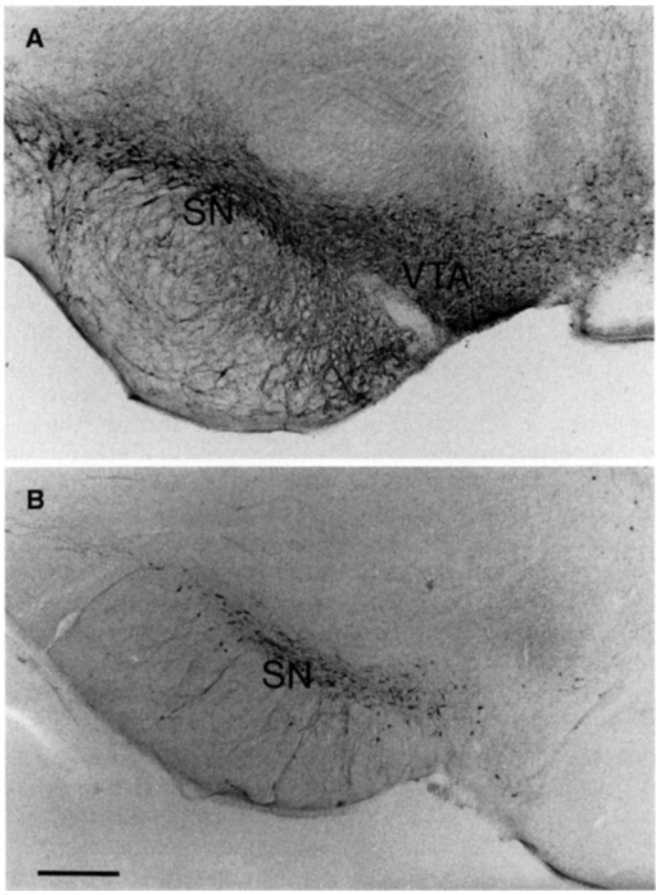

Fig. 1A, B The three mid-brain areas used in this study. **A** Caudal substantia nigra (*SN*) and ventral tegmental area (*VTA*). **B** Rostral SN. *Scale bar* 500 μm

bridge circuit to allow the simultaneous measurement of membrane potential and the injection of current through the microelectrode. Input current and voltage signals were stored on digital audio tapes for subsequent off-line analysis. When an impalement was made, a 0.5–1.0 nA hyperpolarising current was applied for 1–10 min to facilitate the formation of a tight seal.

Electrophysiological analysis

SN pars compacta (SNc) and VTA cells were characterised electrophysiologically in terms of their firing rates, action potential shape, presence of low-threshold Ca^{2+} spike, inward anomalous rectification and outward rectification (Harris et al. 1989; Johnson and North 1992; Llinás et al. 1984; Nedergaard and Greenfield 1992). Input resistance was calculated by measuring the voltage drop to a series of hyperpolarising current pulses in 0.1 nA steps at 1 Hz at rest and during veratrine-induced depolarisation.

Fast-scan cyclic voltammetry and carbon fibre electrodes

In a separate series of experiments, voltammetric measurements were made using a Millar voltammeter (P.D. Systems, West Mosely, UK). Voltammograms were obtained in the simple biphasic mode, with a positive-negative-positive voltage waveform applied to the working electrode (Millar and Williams 1990), with the voltage ramped from 0.0 V to +1.1 V, then to −0.5 V and again back to 0.0 V vs Ag/AgCl. The instrument was used as a three-electrode potentiostat, with an Ag/AgCl wire as the reference electrode and the stainless steel flow outlet of the bath as the auxiliary. Initially, a second carbon fibre microelectrode was used as a "micro-reference" (Millar and Williams 1990), but no improvement in voltammetric response was seen, so this practice was not continued.

The Millar voltammeter offers several possible sampling protocols. In these experiments, the best sensitivity for DA was obtained using a dual waveform setting with two identical scans, separated by 20 ms, generated at a frequency of 2 or 4 Hz. Scan rate in this mode was 660 V/s. Between scan epochs, the electrode was switched out of circuit (Millar and Williams 1990). All current records illustrated here are from the first of the dual scans with background current subtracted electronically.

Carbon fibre microelectrodes, prepared from 7 to 8 μm carbon fibres (Type HM, unsized, Courtaulds) in a 2 mm glass capillary pulled to a 10 μm taper, were a gift from Dr. Julian Millar. The part of the fibre that extended beyond the glass insulation was spark-etched to a length of 20–50 μm, with a tip diameter of 2–4 μm. Electrodes were calibrated in the recording chamber, with 0.5–5 μM DA or 5-HT, or 50 μM 3,4-dihydroxyphenylacetic acid (DOPAC), in Ringer's solution at 32° C, after every two or three slices. Calibration solutions were freshly made immediately before each use. Electrode calibration was very stable throughout a series of slice measurements, after an initial decrease of about 20% in electrode sensitivity upon exposure to a brain slice. The electrodes were 5–8 times more sensitive to 5-HT than to DA and 60–80 times more sensitive to DA than to DOPAC.

Background subtracted voltammograms were monitored continuously on a Gould 1602 storage oscilloscope. Each stimulation experiment and calibration was also recorded on digital audio tape for later analysis.

High-performance liquid chromatography

Slices prepared as above were further dissected to isolate the rostral and caudal SN (including both pars compacta and pars reticulata) and the VTA. DA and DOPAC were extracted using a modification of previously described methods (Anton and Sayre 1962; Felice et al. 1978). Samples were homogenised in 3 ml ice-cold 0.1 M perchloric acid containing 4.3 mM EDTA and 1.6 mM reduced glutathione and then centrifuged (4000 *g*; 0° C; 30 min). Al_2O_3 (80 mg) was added to 2 ml of the supernatant, with 10 nM α-Me-DOPA included as an internal standard: 2 ml 3 M TRIS buffer (pH 8.6) was then added whilst stirring vigorously. This was mixed for 10 min, after which the supernatant was removed and the Al_2O_3 washed twice with 2 ml de-ionised water. DA, DOPAC and α-Me-DOPA were eluted with 400 μl of a solution of 0.25 M boric acid and 0.125 M citric acid. Samples were then run on HPLC using an Apex octadecyl 5 μm reverse phase column (Jones Chromatography) and the following mobile phase: 13.5 mM citric acid, 31.5 mM potassium dihydrogen orthophosphate, 2 mM 1-octane sulphonic acid, 0.2 mM EDTA. An electrochemical detector (Waters 460) was used and concentrations were determined using an integrator (Spectra Physics Analytical SP4400).

Solutions

All drugs and neurochemicals were obtained from Sigma. Ca^{2+}-free Ringer was the same as the modified Ringer's solution except that $CaCl_2$ was excluded, $MgCl_2$ was increased to 10 mM, and 1 mM EGTA was included.

Statistical analysis

All data are expressed as mean ± SEM and, unless otherwise stated in the text, were analysed with the Kruskal-Wallis analysis of variance, followed by post hoc Mann-Whitney *U*-tests.

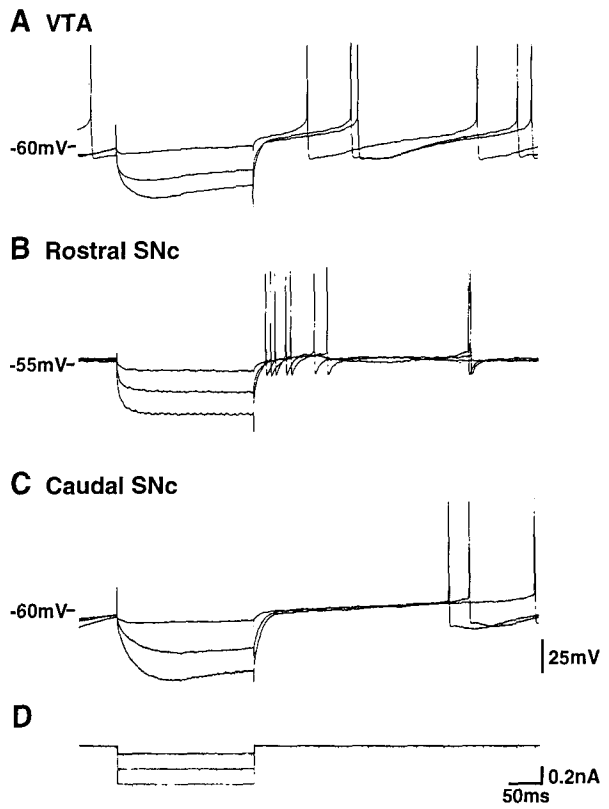

Fig. 2A–D The three cell types recorded in this study. **A** VTA. **B** Rostral SNc (phasic neuron). **C** Caudal SNc (rhythmic neuron). Traces show superimposition of membrane voltage responses to injection of hyperpolarising current pulses (**D**; 0.1, 0.3 and 0.5 nA). Note the similarity of the two cell types shown in **A** and **C**; both have large after-hyperpolarisations (AHPs) and inward and outward rectification. In contrast **B** has a less marked AHP, lacks rectification, and exhibits a low-threshold Ca^{2+} current (LTSg-Ca^{2+})

Results

Electrophysiology

The electrophysiological characteristics of cells recorded from the VTA, caudal SNc ('rhythmic cells') and rostral SNc ('phasic cells') are shown in Fig. 2 and Table 1. Cells recorded from the VTA and caudal SNc were indistinguishable with respect to action potential duration and shape, firing frequency and pattern, membrane potential and input resistance. Cells from the rostral SNc displayed completely different properties, as described earlier (Nedergaard and Greenfield 1992). In brief, the action potential duration was shorter, the after-hyperpolarisation (AHP), although still marked, was smaller, the cells fired in a more phasic (less regular) fashion, and the cells possessed a low-threshold Ca^{2+} spike, activated from hyperpolarised potentials (Llinás et al. 1984).

In order to exclude the possibility that transmitter release following veratrine application for 40 s was due to cell death, veratrine was applied to a total of 21 slices during intracellular recording (5 from the VTA, 9 from the caudal SNc, and 7 from the rostral SNc). The effect of veratrine was seen as a marked depolarisation (latency less than 1 min) accompanied by a decrease in input resistance and spike inactivation (not shown). This effect was reversed completely 18–37 min after the application.

Table 1 Electrophysiological characteristics of neurons from the three areas studied. Action potential width and height and after-hyperpolarisation (*AHP*) size are all measured from threshold. Values shown are mean ± SEM; n = number of slices used in this study

	n	Firing (Hz)	Action potential width (ms)	Action potential height (mV)	AHP size (mV)
VTA	5	2.6 ± 0.7	2.04 ± 0.14	55.3 ± 3.1	30.7 ± 2.9
Rostral SNc	7	9.1 ± 2.3	1.02 ± 0.09**	53.1 ± 2.0	21.4 ± 0.8*
Caudal SNc	9	2.4 ± 0.4	2.00 ± 0.17	54.0 ± 2.6	27.2 ± 1.8

* Significantly different from VTA and caudal SNc, $P < 0.02$
** Significantly different from VTA and caudal SNc, $P < 0.005$

Table 2 Summary of dopamine and 5-hydroxytryptamine responses in rostral and caudal substantia nigra and ventral tegmental area

Region	Number of slices	Number of slices responding (% of total)			
		DA	DA + 5-HT	5-HT	None
Control					
VTA	16	7 (44%)	5 (31%)	2 (13%)	2 (13%)
Rostral SNc	17	1 (6%)	3 (18%)	6 (35%)	7 (41%)
Caudal SNc	10	6 (60%)	1 (10%)	2 (20%)	1 (10%)
Reserpine					
VTA	4	1 (25%)	1 (25%)	0	2 (50%)
Rostral SNc	3	0	0	0	3 (100%)
Caudal SNc	3	0	0	0	3 (100%)

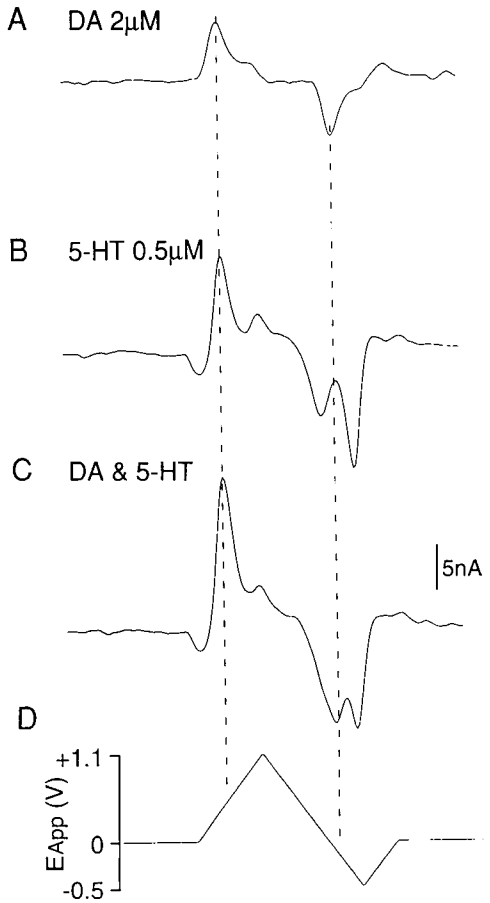

Fig. 3A–D Voltammograms for dopamine (DA) and serotonin (5-HT) calibration solutions and the applied voltage waveform. **A** DA calibration voltammogram. The potential of the DA oxidation peak was +470 mV and that for the reduction peak was −120 mV. **B** 5-HT calibration voltammogram. The 5-HT oxidation peak potential was +470 mV, like that for DA, but its two reduction peaks at +50 mV and −450 mV gave a characteristic voltammogram that is distinct from DA. Electrodes were 5–8 times more sensitive to 5-HT than to DA, probably because of 5-HT adsorption to the electrode surface. For this electrode, calibration factors were 0.31 μM/nA for DA and 0.046 μM/nA for 5-HT. **C** Mixed voltammogram of DA (2 μM) and 5-HT (5 μM). The response reflected the summation of the individual records. **D** The voltage waveform was triphasic, beginning at 0.0 V, with a maximum at +1.1 V and a minimum at −0.5 V, then returning to 0.0 V and out of circuit. Scan rate was about 660 V/s. All peak potentials are given with respect to the Ag/AgCl reference potential. Anodic current is displayed as positive, reduction current as negative in these and other records, unless noted otherwise. Calibration voltammograms were taken in the recording chamber in Ringer's solution at 32° C

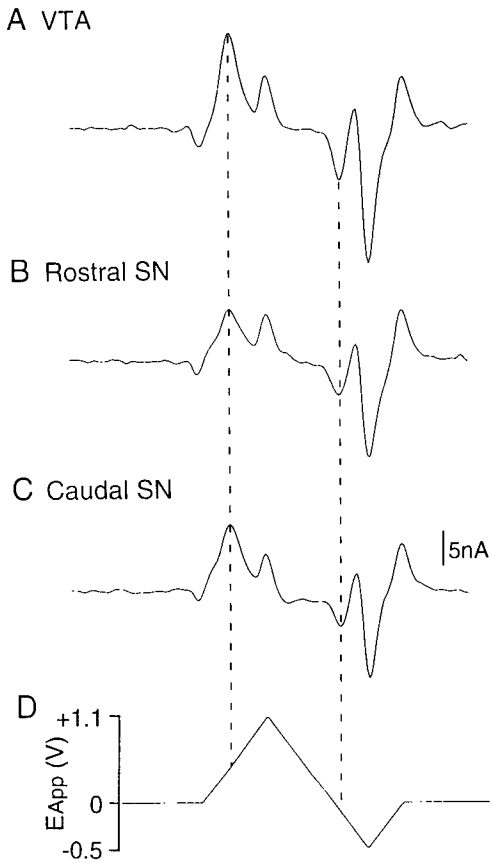

Fig. 4A–D Voltammograms of somatodendritic DA released from VTA and rostral and caudal SN by veratrine. The *dashed vertical lines* indicate the potentials for DA oxidation (+470 mV) and reduction (−160 mV) for comparison with the records taken in the slice. Characterisation of a release signal as DA (Table 2) required that the peak potentials in the slice correspond exactly with those of the corresponding DA calibration voltammogram. **A** The largest DA release signals, 2–5 μM, were recorded in the VTA, which had the highest DA content (Fig. 8) and greatest density of tyrosine hydroxylase-positive cells (Fig. 1). DA calibration factor was 0.34 μM/nA. **B** Rostral and **C** caudal SN pars compacta regions also showed identifiable DA release. This was important confirmation that the level considered to the rostral SNc was indeed dopaminergic. DA calibration factor was 0.27 μM/nA for **B** and 0.33 μM/nA for **C**. **D** The voltage waveform was the same as in Fig. 3

Voltammetric identification of DA and 5-HT

Voltammograms of DA were distinctly different from those of 5-HT (Fig. 3A, B; see also Bull et al. 1990; O'Connor and Kruk 1991; Stamford et al. 1990). While the oxidation peak potential was about +0.5 V vs Ag/AgCl for both compounds, the reduction responses differed. The DA reduction process resulted in a single peak at about −0.15 V (Fig. 3A), whereas that for 5-HT produced two reduction peaks (Fig. 3B), each at a potential distinct from that for DA. By careful comparison of the oxidation and reduction potentials of voltammetric response in mid-brain slices, we could identify when stimulation resulted in the release of DA, or 5-HT, or a combination of the two (Fig. 3C), or of neither substance.

Dendritic release of DA

Of the mid-brain regions examined, the VTA gave responses that were the most reliable and attributable exclusively to DA release following veratrine stimulation (Fig. 4A). This observation is consistent with the dense distribution of DA in this region, compared with the substantia nigra (Fig. 1). In 75% of VTA slices tested (Table 2), the initial phase of the stimulated response was unambiguously attributable to DA release, which could be represented as a classical cyclic voltammogram

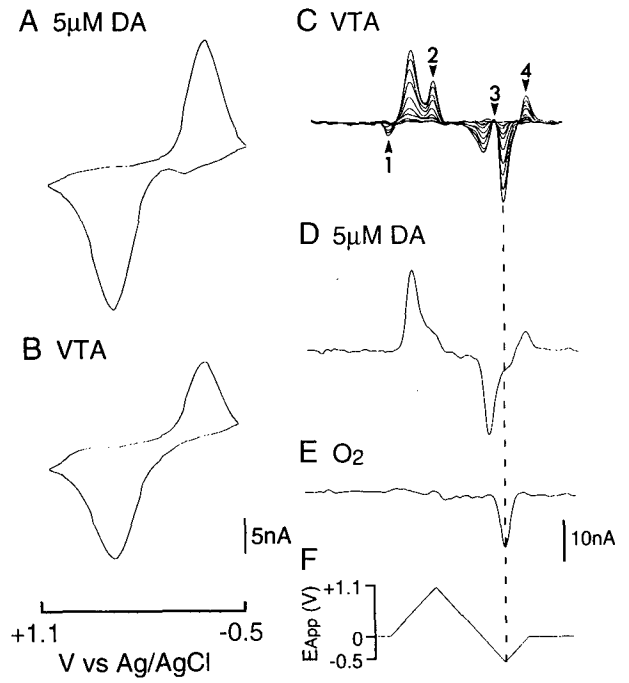

Fig. 5A–F Interpretation of the DA voltammograms recorded in DA somatodendritic regions. **A** DA current-voltage response represented as a classic cyclic voltammogram, with the oxidative peak current negative and the reduction current peak positive. This can be compared with the voltammogram for 5 μM DA in **D** (calibration factor was 0.28 μM/nA). **B** The relatively large DA release responses from the VTA could be represented as cyclic voltammograms. **C** DA release records from the VTA, plotted at 1 s intervals from the commencement of DA detection. These records demonstrate the temporal resolution of FCV, but also show the progressive distortion of an initially clean DA response (oxidation peak: +500 mV, reduction peak: −120 mV) by background shifts. The DA calibration factor was 0.40 μM/nA. The *arrowheads* indicate background currents that presumably result from capacitative changes at the electrode surface/solution interface during tissue depolarisation (see Results). *Arrowheads* 1,2 and 4 reflect points where the direction of the voltage scan was started, stopped or reversed. The artefact at *arrowhead* 3 is near, but apparently unrelated to a reversal point. This positive-going artefact (3) caused the size of the DA reduction peak to be smaller than expected from calibration with 5 μM DA (**D**). **E** Voltammogram of O_2 reduction (about −500 mV) when Ringer's solution was bubbled with 95% O_2/5% CO_2 after previous equilibration with 20% O_2/5% CO_2/75% N_2. The coincidence of the potential of the large reduction peak between *arrowheads* 3 and 4 in **C** with that for O_2 reduction suggested that this peak reflects an increase in O_2 tension in the slice during veratrine-induced depolarisation. This O_2 peak occurred near a reversal potential and probably masked a coincident capacitative artefact. **F** The voltage waveform was the same as in Fig. 3

for DA (Fig. 5A, B). Extracellular DA levels rose to 1–5 μM during this period. A few seconds later, however, shifts in the background (Fig. 5C) or delayed 5-HT signal (see below) contaminated this relatively clean response.

DA release recorded in the VTA rose steadily for 30–45 s after the application of veratrine (Fig. 5C, plotted with an interval of 1 s). These records (Fig. 5C) illustrate the superior time resolution of FCV recording, compared with microdialysis, push-pull, or superfusion systems. The traces also indicate the progressive distortion of the DA voltammogram (compare Fig. 5C and 5D) by changes in background currents that occur at several points on the current record (Fig. 5C, arrowheads). These additional peaks generally corresponded to points on the voltage waveform (E_{app}) at which the voltage was initially applied to the electrode, or the direction of the scan reversed. These so-called switching artefacts are the result of capacitative current flow that reflects electrode surface properties and the external microenvironment of that surface. Normally, these are part of the constant background current that is subtracted from calibration records. In tissue, however, impedance and extracellular ionic composition can change dynamically. Consequently, under depolarising conditions, an altered background may accompany Faradaic oxidation and reduction peaks for DA or other released electroactive species. For this and other reasons (Weidemann et al. 1990), FCV is best suited to study rapid, transient changes in neurotransmitter concentration, rather than to monitor more long-term release processes.

One known "contaminant" of stimulated release signals was a reduction peak that occurred at about −0.5 V and corresponded to changes in O_2 tension (Fig. 5C, E). A consistent observation in all slices was that veratrine-induced depolarisation apparently caused a large increase in PO_2 in the tissue, possibly reflecting an inhibition of metabolism during stimulation. Both increases and decreases in PO_2 have been reported previously when FCV was used to monitor DA and O_2 simultaneously (Kennedy et al. 1992; Zimmerman and Wightman 1991). Because PO_2 was not a focus of the present study, absolute levels and changes were not determined. Another potential source of interference arises from the changes in extracellular pH that accompany tissue stimulation (Chestler 1990). An increase in acidity, for example, is reflected in a background shift that appears as a peak about 200 mV before the DA oxidation peak (Jones et al. 1994; Rice and Nicholson 1989). With the large DA increases seen with veratrine-induced depolarisation in the present study, however, interference from pH shifts was negligible, e.g. as a shoulder before the DA peak (Fig. 4B, C).

Stimulated DA release was also detected in rostral and caudal SN (Fig. 4B, C). While larger somato-dendritic release was evoked from the pars compacta of both regions, DA release could also be detected in the pars reticulata of the caudal SN. Responses attributable to DA release exclusively were elicited in 70% of caudal SN slices and in 25% of rostral slices (Table 2). Stimulated extracellular DA concentrations were often less than 1 μM and were consistently lower than in the VTA. It should be noted that the detection limit for DA in these studies was considered to be about 0.2–0.5 μM. Consequently, a negative response is still consistent with release levels below this limit, as well as the possibility of missing a release site because of the spatially restricted sampling region (less than 10 μm) of the electrode tip.

Indeed, in some experiments a small shift in position of the electrode tip caused a marked change in the amplitude of the DA signal. As in VTA, responses attributable to 5-HT sometimes followed an initial DA increase, especially in the rostral SN.

Detection of 5-HT release

In 30–50% of slices tested, the release of 5-HT was detected either as the primary substance released (Fig. 6) or mixed with DA (Table 2). In most cases, levels of released 5-HT were lower than those of DA. When the response was mixed, DA could be identified initially, followed by the appearance of 5-HT. Whether this sequence of events had functional significance, perhaps reflecting 5-HT diffusion from a distant release site, could not be discerned from the present measurements, because the electrode response to 5-HT was also somewhat slower than to DA during calibration, possibly from the adsorption of 5-HT onto the electrode surface. The serotoninergic response was most pronounced in the rostral SN, with over 50% of the slices tested showing release (Table 2).

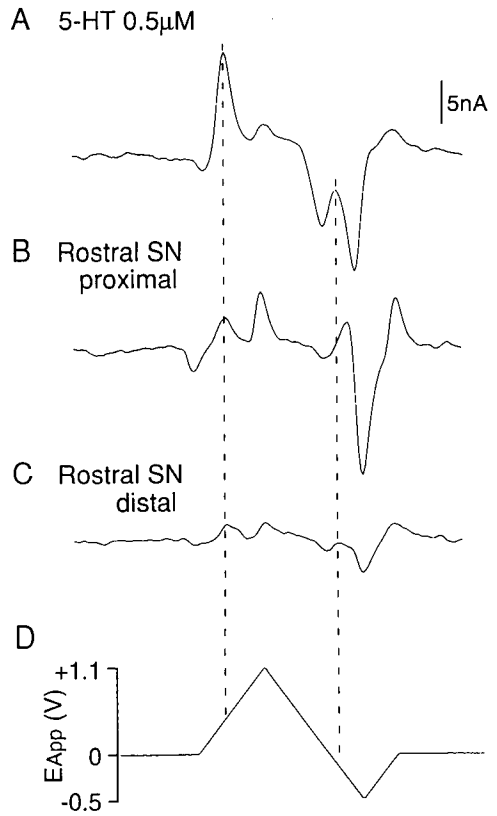

Fig. 6A–D 5-HT release in rostral SN pars reticulata. **A** Calibration voltammogram for 5-HT. Oxidation peak: +470 mV; reduction peaks: +50 mV and −450 mV. The *dashed lines* indicate the position of DA oxidation (+470 mV) and reduction (−120 mV) peaks for this electrode. Calibration factor was 0.045 μM/nA for records **A–C**. **B** 5-HT signal from the region of the proximal dendrites of the rostral SN, near the pars compacta. **C** Slightly smaller 5-HT release response was seen in the distal dendritic region. The greater sensitivity of the electrodes for 5-HT compared with DA permitted detection limits of less than 100 nM for 5-HT, as in **C**. These two records were obtained (in different slices) from subregions of the SN that were separated by only 50 μm. This demonstrates the spatial resolution of FCV with carbon fibre microelectrodes to study discrete sites of biogenic amine release. Because the emphasis of the present study was to compare release of DA and 5-HT among different mid-brain regions, further exploration of subregional differences was not attempted. **D** The voltage waveform was the same as in Fig. 3

Verification of the voltammetric responses

To confirm that the release signals represented detection of DA and 5-HT and were not a measurement artefact, such as a background shift from a change in pH, we repeated the protocol using veratrine depolarisation in the molecular layer of slices from the cerebellum, which has little aminergic input and low monoamine content (Hökfelt and Fuxe 1969; Rice and Nicholson 1987). No catecholamine-related peaks ($n=4$) were detected in the cerebellum (Fig. 7A). Some norepinephrine release might have been anticipated (Bickford-Wimer et al. 1991), but if present was apparently below our detection limits. By contrast, substantial DA release was detected (not illustrated) in slices of striatum, which has a dense dopaminergic innervation.

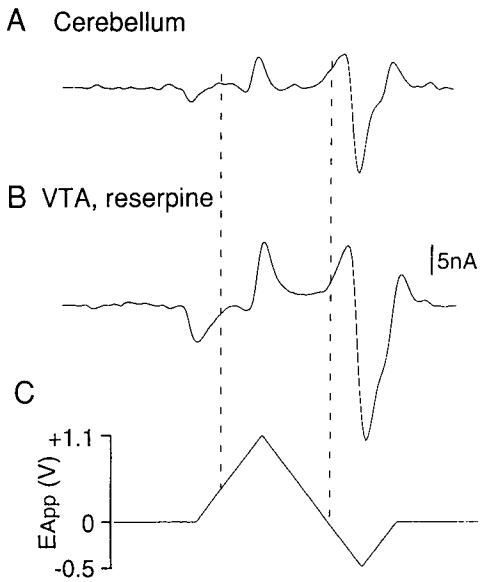

Fig. 7A–C Background shifts alone were recorded during veratrine-induced depolarisation in the DA-poor and 5-HT-poor cerebellum and in the VTA after pretreatment with reserpine. **A** No evidence for biogenic amine release was seen in the molecular layer of cerebellar slices ($n=4$). The *dashed lines* indicate the position of DA oxidation (+530 mV) and reduction (−160 mV) peaks during post calibration of the electrode. DA calibration factor was 0.33 μM/nA. **B** Reserpine pretreatment reduced DA content of the VTA (Fig. 8) and eliminated a DA release signal in 50% of VTA slices tested ($n=4$, Table 2). No release was seen in either rostral or caudal SN after reserpine (Table 2). The elimination of the release response in DA-depleted tissue confirmed that the source of the signal was DA, not an electrode artefact. **C** The voltage waveform is the same as in Fig. 3

DA release could be completely eliminated in mid-brain slices from animals treated with reserpine (6 mg/kg injected i.p. 18 h prior to death) to deplete DA stores (Fig. 7B). No DA (or 5-HT) release was seen in rostral or caudal slices of SN after reserpine (Table 2), although release was sometimes elicited from VTA in depleted animals (Table 2).

HPLC measurement of DA content of mid-brain regions

Total tissue content of DA and DOPAC was determined for rostral and caudal SNc and VTA from control and reserpine-pretreated animals. The results for DA are summarised in Fig. 8. DA levels in the VTA were significantly higher than in either of the other two areas (Kruskal-Wallis analysis of variance, $H = 7.28$, $df = 2$, $P < 0.05$). Reserpine pretreatment significantly reduced DA levels to approximately 25% of control values in both SNc areas (Mann-Whitney U-test, $P < 0.01$) but had little effect in the VTA, where levels remained comparable with, if not higher than, untreated SNc. There was no difference between rostral SNc and caudal SNc either before or after reserpine treatment.

Although extracellular DOPAC was expected to be washed out of slices during superfusion in vitro, high residual levels could have been a major interferent in our measurements of DA. Levels of DOPAC in unincubated slices, however, were found to be 5–10 times less than those of DA in all three brain regions in this study. Total tissue content of DOPAC in the SN was about 10 pmol/mg protein, which can be estimated to correspond to an average tissue concentration of roughly 1 μM. Since the selectivity of the electrodes for DA over DOPAC was 60–80:1, it would require an increase of DOPAC of 60–80 μM to mimic an apparent DA response of 1 μM, which was impossible. Furthermore, DOPAC levels were virtually unchanged after reserpine pretreatment, yet the signals were reduced or eliminated. More conclusively, release signals were unaltered in preliminary studies with slices in which DOPAC formation was inhibited by inclusion of pargyline (10 μM) in preincubation media (S. Cragg, unpublished data). Previous studies have shown that 95% of another potential interferent, ascorbate, is lost from guinea-pig mid-brain slices during incubation (Rice and Cammack 1991). Consequently our FCV measurements were virtually free of DOPAC and ascorbate contributions.

Ca^{2+}-dependence of DA release

The Ca^{2+}-dependence of DA release was examined in VTA slices preincubated for 2–3 h in Ca^{2+}-free media with 1 mM EGTA and 10 mM Mg^{2+}. DA release by veratrine-induced depolarisation was strongly Ca^{2+}-dependent in that release was virtually eliminated in Ca^{2+}-free media (Fig. 9; $n = 5$). Several other Ca^{2+} channel blockers were tested, including Mn^{2+} and Co^{2+}, but these proved to be electroactive or otherwise interfered with the measurement, so that they could not be used.

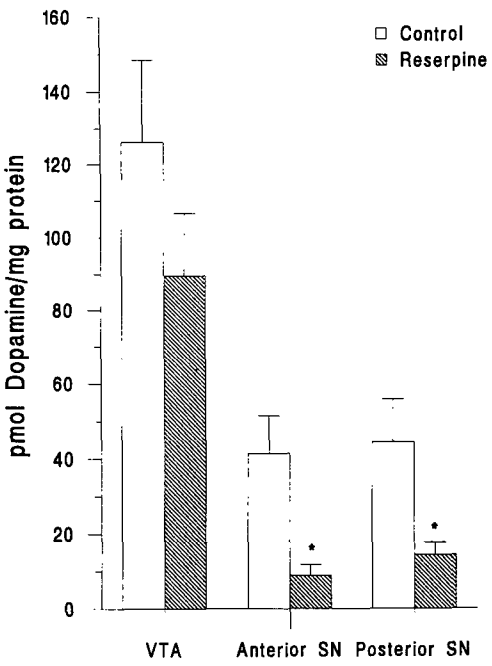

Fig. 8 Effect of reserpine on mid-brain DA levels. Reserpine (6 mg/kg) was administered i.p. 18 h before death. Levels were measured on HPLC (see Methods). For each group $n = 5$. * Significantly different from control ($P < 0.01$)

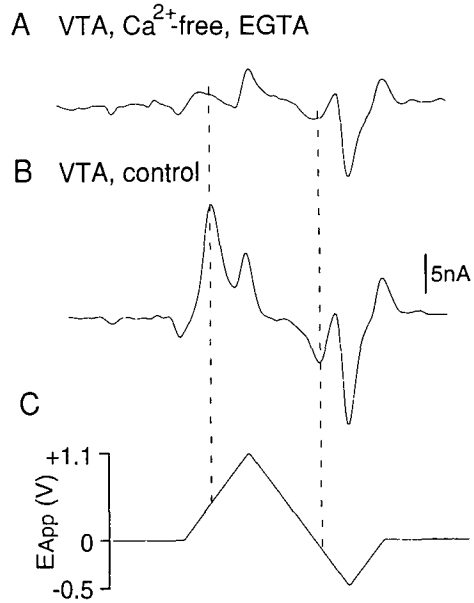

Fig. 9A–C Somatodendritic release of DA was Ca^{2+}-dependent. **A** DA release from the VTA was completely inhibited in Ca^{2+}-free media with added EGTA, which demonstrated the Ca^{2+}-dependence of the release process. **B** Control release response from an adjacent VTA slice. The concentration of DA attained in the control tissue was over 5 μM (calibration factor was 0.4 μM/nA). The *dashed lines* indicate the position of DA oxidation (+530 mV) and reduction (−160 mV) peaks during post calibration of the electrode. **C** The voltage waveform was the same as in Fig. 3

Discussion

Heterogeneity of mid-brain neurons

Although the VTA is a region anatomically distinct from the SN, the DA cells in the two nuclei are frequently referred to as a single population of mid-brain neurons. The homogeneity of electroresponsiveness of VTA and caudal SN cells in the present study (Fig. 2 and Table 1) corresponds to previous findings (Johnson and North 1992). By contrast, however, there is heterogeneity of electroresponsiveness between neurons in the rostral SNc and those in the VTA and caudal SNc (Murphy and Greenfield 1992; Nedergaard and Greenfield 1992). We have monitored directly the extracellular concentration of DA released with veratrine-induced depolarisation in each of these mid-brain DA cell body regions. In agreement with the relative densities of TH staining (Fig. 1), release was higher in VTA than either rostral or caudal SN. Both rostral and caudal SN showed a positive immunochemical reaction to tyrosine hydroxylase (Fig. 1), had similar DA content, indicated by HPLC analysis (Fig. 8), and had releasable pools of DA, as seen in the voltammetric studies (Fig. 4B, C). These data are consistent with the hypothesis that rostral, as well as caudal cells are dopaminergic.

Relative advantages and limitations of fast-scan cyclic voltammetry

As mentioned in the Introduction, the spatial resolution of both the push-pull cannulae technique and microdialysis is in excess of 1 mm, while the temporal resolution afforded by either technique, when coupled with a radiolabelled assay or HPLC, is 10–15 min. It is clear, therefore, that neither of these more established systems would be able to differentiate reliably between DA released from the VTA and the adjacent SN, nor between rostral and caudal SN as defined here. By contrast, FCV, used in the present study, permitted direct, quantitative detection of DA release with sub-second sampling frequency, as reported previously for striatum (Kuhr and Wightman 1986; Stamford et al. 1986). Furthermore, the spatial resolution of carbon fibre microelectrodes permitted detection of regional differences in the size of a DA release signal when the electrode was moved among the three adjacent areas of mid-brain. Hence the superior time-space resolution of direct voltammetric detection of DA was vindicated for studying endogenous relase in the SN.

The electrochemical nature of the analysis process, however, limited the choice of secretagogues that could be used. Agents such as the DA uptake blocker nomifensine, which has been used in both push-pull cannulae perfusion and microdialysis, is electroactive at an interfering potential. Potassium, while not electroactive, caused interfering background shifts (possibly from activation of tissue metabolic pathways), which distorted the response. Veratrine, on the other hand, produced a fast release signal, which was much cleaner than that seen with potassium. The primary action of veratrine is to enhance the opening of sodium channels, which are in turn blocked by TTX (Tagerud and Cuello 1979). For this reason the effects of TTX on endogenous DA release were not explored in this study.

Nonetheless, veratrine proved an efficient means of evoking DA release and was shown by intracellular recordings to have a marked but reversible depolarising action. Furthermore, the data suggest that the release process was physiologically relevant since it was Ca^{2+}-dependent (Fig. 9), as already shown in acute preparations using push-pull cannulae (Cheramy et al. 1981) and microdialysis (Kalivas and Duffy 1991).

Detection of endogenous DA and 5-HT release

The major contributor to the evoked release response was DA, as indicated by the similarity of the recorded voltammograms to that of DA in calibration. This signal was readily distinguishable from 5-HT. Nonetheless, it was not surprising that 5-HT was also detectable, since the SN receives a major serotoninergic input from the Raphé nuclei (Dray et al. 1976; Fibiger and Miller 1977) and Ca^{2+}-dependent 5-HT release from SN has been reported previously (Hery et al. 1980; Reubi and Emson 1978). Cleaner DA release signals could be obtained in 5-HT-lesioned animals (S. Cragg, unpublished data), but we did not find an advantage in this protocol, since DA and 5-HT could be distinguished voltammetrically (Fig. 3). It is interesting to note that, although a variety of possible interactions between 5-HT and DA are likely to occur in mid-brain DA regions, previous studies have found that the level of potassium-stimulated DA release in the SN was unaltered by serotoninergic lesions (Tagerud and Cuello 1979).

Serotoninergic terminals can make direct contact with dopaminergic dendrites within the pars reticulata (Nedergaard et al. 1988). Indeed, it is noteworthy that a serotoninergic signal was most frequently observed in the rostral SN, where exogenous 5-HT has already been shown to have a site-selective action on a dendritic calcium conductance (Nedergaard et al. 1988). In this previous study, there was a clear variation in the potency of 5-HT depending on whether it was applied to the distal or proximal sections of the dendrites of rostral nigral neurons. The present observations, such as those in Fig. 6B, C, illustrate that carbon fibre electrodes used with FCV have sufficient spatial resolution to discriminate transmitter release in proximal versus distal dendritic zones. On the other hand, a much more extensive study of regional release sites would have to be conducted to determine the relationship between these voltammetric findings and the earlier report (Nedergaard et al. 1988) on the actions of iontophoretically-applied 5-HT.

It is unlikely that the catecholamine voltammograms obtained were the result of some inherent artefact rather

than endogenous DA, since the signal varied both within and among the three regions of mid-brain. Furthermore, in the cerebellum,where there is a paucity of catecholamines, no signal was observed at all. Within the SN there was no signal in tissue treated with reserpine, a drug classically associated with a complete disruption of DA storage (Björklund and Lindvall 1975). However, more recent evidence suggests that, within the SN particularly, depletion is much less dramatic following reserpine treatment than in the striatum: tissue levels are back to 40% within 12 h and indeed, maximal depletion is only up to 17% of control values (Elverfors and Nissbrandt 1991). These findings are consistent with those in this study using HPLC (Fig. 8). The fact that release of DA was sometimes detectable from the VTA after reserpine is attributable to the residual levels of DA that were actually similar to the DA content of the non-reserpinised SN (Fig. 8). Nonetheless, the finding that within the two regions of the SN itself no release was detectable after reserpine treatment, and that the probability of detecting release in the VTA was reduced after reserpine, supports the conclusion that the observations represented depolarisation-dependent release of DA.

Significance of dendritic DA release

Although DA release from terminals in the striatum and from dendrites in the SN are both Ca^{2+}-dependent and depolarisation-dependent, data from the striatum cannot be extrapolated a priori to the SN in order to describe the process of dendritic release. Simultaneous comparison of release in SN and striatum shows differences with respect to the role of SNc action potentials in the release process (Cheramy et al. 1981; Kalivas and Duffy 1991). These data, combined with electrophysiological observations in SNc (Nedergaard et al. 1988) have prompted the idea that dendritic release of DA might arise through local dendritic activation from synaptic input, rather than through antidromic invasion of the dendrites by action potentials generated in the soma. The mechanism of release remains open to speculation.

Possible functional roles for dendritically released DA, on the other hand, are becoming clearer. Before dendritic release was actually demonstrated, it was proposed that such a process might serve as a form of negative feedback for nigrostriatal cells (Groves et al. 1975), since the DA-releasing agent amphetamine inhibited nigral cell firing in SNc. More recent physiological studies have led to the suggestion that DA-mediated responses might act as a low-pass filter for incoming synaptic transmission (Greenfield 1985). A putative mechanism of action could arise from dopaminergic modulation of transmitter release from pre-synaptic terminals. For example, nigral application of DA has been shown to alter the release of 5-HT in SN (Hery et al. 1980). This is particularly intriguing in light of the effect of 5-HT on excitability in DA cells and dendrites (Nedergaard et al. 1988, 1991) and suggests the possibility of intricate patterns of co-modulation. Similarly, DA facilitates the release of GABA (Reubi et al. 1977) in the SN. This appears to have functional correlates, since it was recently shown that DA enhances $GABA_b$ receptor-mediated post-synaptic potentials via D1 receptor activation in DA cells of the VTA (Cameron and Williams 1993).

The data presented here describe the first direct monitoring of DA and 5-HT release in mid-brain DA regions. These results indicate that dendritic DA release is not uniform throughout the mid-brain. Levels of release correlated well with DA content, with higher levels of content and release in VTA than in either region of the SN. Intriguingly, DA release was similar in rostral and caudal SN, despite the distinctly different electroresponsiveness of cells in each region. Taken together, these data suggest that dendritically released DA could have regionally distinct functions, depending on its concentration in the extracellular fluid and on the properties of local cells. This report opens a window for future studies exploiting the high time-space resolution of FCV in order to probe the mechanism of dendritic release and further elucidate its functional value.

Acknowledgements This work was funded by Bristol-Myers Squibb Co. (USA). Additional support to M.E.R. (NS-28480) and C.N. (NS-28642) was provided by the NIH. S.N. holds a grant from the Danish MRC. C.D.R. holds an MRC studentship. The authors would like to thank PD Systems Ltd., West Mosely, UK for the generous loan of the Millar voltammeter and Dr. Julian Millar for the gift of the carbon fibre electrodes. We should also like to thank Ms P.M. Cordery for preparation of the histology, Messrs. M. Preston, C.P. Webb and M. Bertoz for excellent electronic and technical assistance, and Ms. S. Cragg for unpublished material relating to 5-HT lesions and pargyline administration.

References

Adams RN (1990) In vivo electrochemical measurements in the CNS. Prog Neurobiol 35:297–311

Anton AH, Sayre DF (1962) A study of the factors affecting the aluminium oxide-trihydroxyindole procedure for the analysis of catecholamines. J Pharmacol Exp Ther 138:360–375

Armstrong-James M, Fox K, Kruk ZL, Millar J (1981) Quantitative iontophoresis of catecholamines using multibarrel carbon fibre microelectrodes. J Neurosci Methods 4:385–406

Bickford-Wimer P, Pang K, Rose G, Gerhardt GA (1991) Electrically-evoked release of norepinephrine in the rat cerebellum: an in vivo electrochemical and electrophysiological study. Brain Res 558:305–311

Björklund A, Lindvall O (1975) Dopamine in dendrites of substantia nigra neurons: suggestions for a role in dendritic terminals. Brain Res 83:531–537

Bull DR, Palij P, Sheehan MJ, Millar J, Stamford JA, Kruk ZL, Humphrey PPA (1990) Application of fast-scan cyclic voltammetry to measurement of electrically evoked dopamine overflow from brain slices in vitro. J Neurosci Methods 32:37–44

Cameron DL, Williams JT (1993) Dopamine D1 receptors facilitate transmitter release. Nature 366:344–347

Cheramy A, Leviel V, Glowinski J (1981) Dendritic release of dopamine in the substantia nigra. Nature 289:537–542

Chesler M (1990) The regulation and modulation of pH in the nervous system. Prog Neurobiol 34:401-427

Chiodo LA, Antelman SM, Caggiula AR, Lineberry CG (1980) Sensory stimuli alter the discharge rate of dopamine (DA)

neurons: evidence for two functional types of DA cells in the substantia nigra. Brain Res 189:544–549
Cuello AC (1982) Storage and release of amines, amino acids and peptides from dendrites. Prog Brain Res 55:205–224
Cuello AC, Kelly JS (1977) Electron microscopic autoradiographic localization of [^{3}H]-dopamine in the dendrites of the dopaminergic neurones of the rat substantia nigra in vivo. Br J Pharmacol 59:P527–528
Dray A, Davies J, Oakley NR, Tongroach P, Vellucci S (1976) The dorsal and medial raphe projections to the substantia nigra in rat: electrophysiological, biochemical and behavioural observations. Brain Res 151:431–442
Elverfors A, Nissbrandt H (1991) Reserpine-insensitive dopamine release in the substantia nigra? Brain Res 557:5–12
Elverfors A, Jonason J, Jonason G, Leonsson G, Nissbrandt H (1992) Determination of release of endogenous dopamine from superfused substantia nigra slices. J Neurosci Method 45:209–215
Felice LJ, Felice JD, Kissinger PT (1978) Determination of catecholamines in rat brain parts by reverse-phase ion-pair liquid chromatography. J Neurochem 31:1461–1465
Fibiger HC, Miller JJ (1977) An anatomical and electrophysiological investigation of the serotonergic projection from the dorsal raphe nucleus to the substantia nigra in the rat. Neuroscience 2:975–987
Geffen LB, Jessell TM, Cuello AC, Iversen LL (1976) Release of dopamine from dendrites in rat substantia nigra. Nature 260:258–260
Greenfield SA (1985) The significance of dendritic release of transmitter and protein in the substantia nigra. Neurochem Int 7:887-901
Greenfield SA, Grunewald RA, Foley P, Shaw SG (1983) Origin of various enzymes released from the substantia nigra and caudate nucleus: effects of 6-hydroxydopamine lesions of the nigro-striatal pathway. J Comp Neurol 214:87–92
Groves PM, Wilson CJ, Young SJ, Rebec GV (1975) Self-inhibition by dopaminergic neurons. Science 190:522-529
Harris NC, Webb C, Greenfield SA (1989) A possible pacemaker mechanism in pars compacta neurones of the guinea pig substantia nigra revealed by various ion channel blocking agents. Neuroscience 31:363–370
Hery F, Soubrie P, Bourgoin S, Motastruc JL, Artaud F, Glowinski J (1980) Dopamine released from dendrites in the substantia nigra controls the nigral and striatal release of serotonin. Brain Res 193:143–151
Hökfelt T, Fuxe K (1969) Cerebellar monoamine nerve terminals, a new type of afferent fibre to the cortex cerebelli. Exp Brain Res 9:63–72
Johnson SW, North RA (1992) Two types of neurone in the rat ventral tegmental area and their synaptic inputs. J Physiol (Lond) 450:455–468
Jones SR, Mickelson GE, Collins LB, Kawagoe KT, Wightman RM (1994) Interference by pH and Ca^{2+} ions during measurements of catecholamine release in slices of rat amygdala with fast-scan cyclic voltammetry. J Neurosci Methods (in press)
Juraska JM, Wilson CJ, Groves PM (1977) The substantia nigra of the rat: a Golgi study. J Comp Neurol 172:585–600
Kalivas PW, Duffy P (1991) A comparison of axonal and somatodendritic dopamine release using in vivo dialysis. J Neurochem 56:961–967
Kennedy RT, Jones SR, Wightman RM (1992) Simultaneous measurement of oxygen and dopamine: coupling of oxygen consumption and neurotransmission. Neuroscience 47:603–612
Kuhr W, Wightman RM (1986) Real-time measurement of dopamine release in rat brain. Brain Res 81:168–171
Llinás R, Sugimori M (1980) Electrophysiological properties of in vitro Purkinje cell somata in mammalian cerebellar slices. J Physiol (Lond) 305:171–195
Llinás R, Greenfield SA, Jahnsen H (1984) Electrophysiology of pars compacta cells in the in vitro substantia nigra – a possible mechanism for dendritic release. Brain Res 294:127–132
Luparello TJ (1967) Stereotaxic atlas of the forebrain of the guinea pig. Karger, New York
Mercer L, del Fiacco M, Cuello AC (1978) The smooth endoplasmic reticulum as a possible storage site for dendritic dopamine in substantia nigra neurones. Experientia 35:101–103
Millar J, Williams GV (1990) Fast differential ramp voltammetry: a new voltammetric technique designed specifically for use in neuronal tissue. J Electroanal Chem 282:33–49
Millar J, Stamford JA, Kruk ZL, Wightman RM (1985) Electrochemical, pharmacological and electrophysiological evidence of rapid dopamine release and removal in rat caudate nucleus following electrical stimulation of the median forebrain bundle. Eur J Pharmacol 109:341–348
Murphy KPSJ, Greenfield SA (1992) Neuronal selectivity of ATP-sensitive potassium channels in guinea-pig substantia nigra revealed by responses to anoxia. J Physiol (Lond) 453:167–183
Nedergaard S, Greenfield SA (1992) Sub-populations of pars compacta neurons in the substantia nigra: the significance of qualitatively and quantitatively distinct conductances. Neuroscience 48:423–437
Nedergaard S, Bolam P, Greenfield SA (1988) Facilitation of a dendritic calcium conductance by 5-hydroxytryptamine in the substantia nigra. Nature 333:174–177
Nedergaard S, Flatman JA, Engberg I (1991) Excitation of substantia nigra pars compacta neurones by 5-hydroxytryptamine in vitro. Neuroreport 2:329–332
Nicholson C, Rice ME (1988) Use of ion-selective microelectrodes and voltammetric microsensors to study the brain cell microenvironment. In: Boulton AA, Baker GB, Walz W (eds) Neuromethods, vol 9. The neuronal microenvironment. Humana Press, Clifton, N.J., pp 247–361
Nieoullon A, Cheramy A, Glowinski J (1977) Release of dopamine in vivo from cat substantia nigra. Nature 266:375–377
O'Connor JJ, Kruk ZL (1991) Fast cyclic voltammetry can be used to measure stimulated endogenous 5-hydroxytrypamine release in untreated rat brain slices. J Neurosci Methods 38:25–33
Parizek J, Hassler R, Bak IJ (1971) Light and electron microscopic autoradiography of substantia nigra of rat after intraventricular administration of tritium-labelled norepinephrine, dopamine, serotonin and the precursors. Z Zellforsch Mikrosk Anat 115:137–148
Reubi J-C, Emson PC (1978) Release and distribution of endogenous 5-HT in rat substantia nigra. Brain Res 139:168–174
Reubi J-C, Iverson LL, Jessel TM (1977) Dopamine selectively increases [^{3}H]GABA release from slices of rat substantia nigra *in vitro*. Nature 268:652–654
Rice ME, Cammack J (1991) Anoxia resistant turtle brain maintains ascorbic acid content in vitro. Neurosci Lett 132:141–145
Rice ME, Nicholson C (1987) Interstitial ascorbate in turtle brain is modulated by release and extracellular volume change. J Neurochem 49:1096–1103
Rice ME, Nicholson C (1989) Measurement of nanomolar dopamine diffusion using low-noise perfluorinated ionomer-coated carbon fibre microelectrodes and high-speed cyclic voltammetry. Anal Chem 61:1805–1810
Richards CD, Rice ME, Nedergaard S, Hounsgaard J, Midtgaard J, Nicholson C, Greenfield SA (1993) Somato-dendritic release of dopamine from substantia nigra and VTA monitored in real-time with fast cyclic voltammetry in vitro. Soc Neurosci Abstr 19:739
Robertson GS, Damsma G, Fibiger HC (1991) Characterization of dopamine release in the substantia nigra by in vivo microdialysis in freely moving rats. J Neurosci 11:2209–2215
Santiago M, Westerink BHC (1990) Characterization of the in vivo release of dopamine as recorded by different types of intracerebral microdialysis probes. Naunyn-Schmiedeberg's Arch Pharmacol 342:407–414
Santiago M, Machado A, Cano J (1992) Fast sodium channel dependency of the somatodendritic release of dopamine in the rat's brain. Neurosci Lett 148:145–147

Sotelo C (1971) The fine structural localization of norepinephrine-3H in the substantia nigra and area postrema of the rat. An autoradiographic study. J Ultrastruct Res 36:824–841

Stamford JA, Kruk ZL, Millar J (1986) Sub-second striatal dopamine release measured by in vivo voltammetry. Brain Res 381:351–355

Stamford JA, Kruk ZL, Millar J (1990) Striatal dopamine terminals release serotonin after 5-HTP pretreatment: in vivo voltammetric data. Brain Res 515:173–180

Tagerud SEO, Cuello AC (1979) Dopamine release from the rat substantia nigra in vitro. Effect of raphe lesions and veratrine stimulation. Neuroscience 4:2021–2029

Wassef M, Berod A, Sotelo C (1981) Dopaminergic dendrites in the pars reticulata of the rat substantia nigra and their striatal input. Combined immunocytochemical localization of tyrosine hydroxylase and anterograde degeneration. Neuroscience 6:2125–2139

Weidemann DJ, Basse-Tomusk A, Wilson RL, Rebec GV, Wightman RM (1990) Interference by DOPAC and ascorbate during attempts to measure drug-induced changes in neostriatal dopamine with Nafion-coated, carbon fiber electrodes. J Neurosci Methods 35:9–18

Westerink BHC, Damsma G, Rollema H, de Vries JB, Horn AS (1987) Scope and limitations of in vivo brain dialysis: a comparison of its application to various neurotransmitter systems. Life Sci 41:1763–1776

Wilson CJ, Groves PM, Fifková E (1977) Monoaminergic synapses, including dendro-dendritic synapses in the rat substantia nigra. Exp Brain Res 30:161–174

Zimmerman JB, Wightman RM (1991) Simultaneous electrochemical measurements of oxygen and dopamine in vivo. Anal Chem 63:24–28