Differences in Pharmacological Properties of Dopamine Release Between the Substantia Nigra and Striatum: An In Vivo Electrochemical Study

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ABSTRACT

The properties of dopamine (DA) release in the rat substantia nigra (SN) and striatum were investigated using high-speed chronoamperometric recordings in brain slices. In both brain regions, a 2-min bath superfusion with 30 mM KCl produced robust DA-like electrochemical signals, with the mean amplitude of the signal being 10-fold greater in the striatum than the SN. The reproducibility of the response was confirmed by a second stimulus (S2)/first-stimulus (S1) ratio of >0.8 in both regions. The bath application of tetrodotoxin significantly reduced the S2/S1 ratio in both the striatum and SN, implicating the requirement for voltage-sensitive sodium channels in the DA-release process. However, the application of cadmium chloride, a nonselective blocker of voltage-sensitive calcium channels, reduced the S2/S1 ratio only in the striatum and not within the SN. Moreover, removal of Ca\(^{2+}\) from the buffer did not significantly affect release within the SN, despite a >85% reduction in release within the striatum. In addition, although the D\(_2\) receptor antagonist sulpiride enhanced the S2/S1 ratio in the striatum, no effect of this agent was seen in the SN. Finally, the amplitude of \(d\)-amphetamine-evoked responses, relative to the KCl-evoked release, was much smaller in the striatum than in the SN. Taken together, these data support the hypothesis that differences in the mechanism or mechanisms of release exist between somatodendritic and axonal elements within the nigrostriatal pathway.

Current models of basal ganglia function emphasize the actions of dopamine (DA) in the striatum, where it regulates descending pathways to both the substantia nigra (SN) and globus pallidus (Gerfen, 1992). However, it is believed that DA may also contribute to basal ganglia output through its actions within “extrastriatal” nuclei, especially the SN, where DA cell bodies can synthesize, store, and release DA (Bjorklund and Lindvall, 1975; Cheramy et al., 1981; Chessex et al., 1983; Delfs et al., 1986). Through its actions on D\(_2\) receptors located on DA cell bodies in the SN pars compacta (SNC), somatodendritically released DA can modulate SNC cell firing and subsequent DA output in the striatum (Santiago and Westerink, 1991b). In addition, through modulation of \(\gamma\)-aminobutyric acid and/or glutamate release within the SN pars reticulata, DA may regulate nigral output to the thalamus and cortex (Abarca et al., 1995; Aveyes et al., 1995). The behavioral consequences of DA release within the SN have been demonstrated in at least two ways. First, direct infusion of DA agonists into the SN modulates locomotor activity, both in normal animals (Bradbury et al., 1983; Kelly et al., 1987) and in 6-hydroxydopamine-lesioned rats (LaHoste and Marshall, 1990). Second, the treatment of hemiparkinsonian rats and monkeys with glial cell line-derived neurotrophic factor results in improvements in locomotor behaviors that correlate with a functional enhancement of nigral, but not striatal, DA markers (Hoffer et al., 1994; Gash et al., 1996; Hoffman et al., 1997). Thus, although striatal DA is clearly important in maintaining the normal output of the basal ganglia, DA released within the SN is also critical. Presently, however, there is little known about the mechanisms that govern somatodendritic DA release.

Due to the small size and low tissue DA content of the SN, the measurement of somatodendritic release requires an analytical technique with a sufficient degree of both sensitivity and spatial resolution. In vivo voltammetric recordings thus are ideally suited for such studies, especially in brain slices, where direct visualization of the SN can be achieved. Recent

ABBREVIATIONS: DA, dopamine; SN, substantia nigra; SNC, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; T\(_c\), clearance rate; DAT, dopamine transporter; TTX, tetrodotoxin; aCSF, artificial cerebrospinal fluid; 5-HT, serotonin; red/ox ratio, reduction/oxidation ratio.
voltammetric comparisons of electrically evoked somatodendritic and axonal release of DA were performed by Rice and colleagues; these studies have shown that DA release in the SN differs from release in the striatum, not only in magnitude but also in its regulation by D2 autoreceptors (Cragg and Greenfield, 1997; Rice et al., 1997). Although chemical stimuli, such as d-amphetamine and KCl, have been shown to promote DA release in microdialysis studies (Robertson et al., 1991; Hoffman et al., 1997), these agents have not been directly compared in electrochemical recordings from the striatum and SN. Because KCl and d-amphetamine are believed to promote DA release in a Ca2+-dependent and Ca2+-independent fashion, respectively (Carboni et al., 1989; Westerink et al., 1989), the use of these two stimuli may be helpful in determining the relative contributions of these processes to somatodendritic DA release.

In the present study, we used high-speed chronoamperometric recordings in rat brain slices to compare DA release in the SN with that observed in the striatum in response to two different chemical stimuli. First, a brief application of KCl was used to promote DA release in both brain regions, and different chemical stimuli, such as d-amphetamine, an agent that promotes DA release in microdialysis studies (Robertson et al., 1991; Hoffman et al., 1997), these agents have not been shown to be effective in vivo electrochemistry (IVEC) system (Medical Systems, Inc., Greenvale, NY). The methodology used here has been previously described (Hoffman and Gerhardt, 1998). Briefly, square-wave pulses of 0.00 to +0.55 V, with respect to an Ag/AgCl reference electrode, were applied to the working electrode for 100 ms and repeated 5 times per second. The resulting oxidation current (measured during the 0.50- to 0.55 V step) and reduction current (measured during the +0.55- to 0.5 V step) were digitally integrated during the last 80 ms of each pulse. Single carbon fiber electrodes (100 μm length × 30 μm O.D.) were coated with Nafion using a high-temperature coating procedure (Hoffman and Gerhardt, 1998). All electrodes were calibrated using 2 to 10 μM increments of DA before each experiment. Analysis of a subset (n = 80) of the electrodes used in these recordings determined that electrodes responded in a linear fashion to DA (r2 = 0.998 ± 0.001) and were highly selective (1570 ± 200:1) for DA versus either 3,4-dihydroxyphenylacetic acid or ascorbate. To obtain a signal-to-noise ratio of >3, DA signals had to achieve amplitudes of 26 ± 2 nA. Extracellular changes in DA concentration were expressed quantitatively based on the pre-experiment DA calibration curves (Gerhardt et al., 1984). Calibrations performed in either Ca2+-free aCSF (n = 9) or in PBS with added Ca2+ (n = 10) revealed that neither the addition of nor the removal of Ca2+ significantly affected the sensitivity of the electrodes. In addition, the recording properties of the electrodes were not altered at low pH (6.5) versus pH 7.4 (n = 9).

Electrodes were lowered into the desired recording site or site so that the tip of the recording electrode was positioned at a depth of 100 to 150 μm from the surface of the tissue. Recordings within the SNc were performed in the medial region of the structure, encompassing both the SNc and SN pars reticulata. Recordings within the striatum were performed in the dorsolateral region of the structure, which contains the highest density of DA terminals and uptake sites. After allowing several minutes for the electrode to stabilize in the tissue, KCl or d-amphetamine was applied to the bath to promote DA release. Due to the rapid onset of DA release observed in the presence of KCl, bath applications of KCl were limited to ≤2 min. Although signals typically returned to baseline within 8 to 10 min after KCl application, preliminary data revealed that reproducible signals were obtained after longer intervals (>20 min). Thus, successive KCl stimuli were applied 30 min apart. For d-amphetamine, maximal effects were seen between 5 and 10 min after drug application. All other drugs, applied between successive stimulations, were given at least a 10-min wash-in period.

Confirmation of Reduction/Oxidation (Red/Ox) Ratios. As we previously described (Hoffman et al., 1998), the red/oxy ratio of the electrochemical signal can be used to distinguish between DA and 5-HT. Both DA and 5-HT have been reported to be measured within the rat SN (Rice et al., 1994; Iravani and Kruk, 1997). To further confirm this in the present study, direct superfusion of DA and 5-HT into slices containing the SN was used because this allowed for a systematic variation of 5-HT/DA concentrations. Due to the rapid uptake of DA and 5-HT in slices, high bath concentrations (10–50 μM) were required to observe signals at the electrodes, which were immersed in the tissue. In general, concentrations achieved at the electrode using this approach were ≤1 μM. Once a detectable signal was achieved, the red/oxy ratios were calculated by dividing the reduction current by the oxidation current at the peak of the electrochemical signal.

Signal Parameters. For each signal, the peak amplitude of the oxidation current was used to determine the DA concentration. In experiments involving successive stimuli, measurements were expressed as a ratio of the peak amplitude of the second response (S2) to the peak amplitude of the first response (S1). In addition, the clearance rate (Tc, in micromole per second) was determined on the pseudolinear portion of the signal decay curve, as previously de-
scribed (Cass, 1997; Hoffman and Gerhardt, 1998). The red/ox ratio was also analyzed for each signal. As we recently described, this ratio, which is generally ≥0.5 for DA (Cass, 1997), may achieve values of ≥1 for DA in the brain slice preparation, possibly due to some adsorption of DA to the electrode surface. However, this is readily distinguishable from the red/ox ratio for serotonin (5-HT) which is typically ≤0.1 (Hoffman et al., 1998).

**Statistical Analysis.** Statistical analyses were performed using SigmaStat 2.0 (Jandel Scientific Software, San Rafael, CA). Drug effects on DA release were analyzed using a two-way ANOVA (treatment and brain region as factors) followed by Tukey’s post hoc comparisons. All other statistical comparisons used a two-tailed Student’s t test or a one-way ANOVA followed by Tukey’s post hoc comparisons. In all cases, statistical significance was defined as p < 0.05.

**Results**

**Effects of KCl Superfusion on DA Release**

We previously reported that direct, local application of high KCl via pressure ejection produces DA release in striatal, but not nigral, brain slices (Hoffman et al., 1998). Therefore, superfusion of brain slices was used in the present study to promote DA release. In both the striatum and SN, a 2-min bath application of 30 mM KCl produced readily detectable electrochemical signals that rose to a maximal amplitude and decayed to baseline levels within 4 to 5 min. Peak amplitudes in individual recordings varied, in both the SN (range, 0.08–1.22 μM) and the striatum (range, 0.41–13.66 μM), perhaps due to the heterogeneity of release sites. As shown in Table 1, the mean peak amplitude of the signals was significantly (p < 0.05, Student’s two-tailed t test) higher within the striatum than in the SN, consistent with the higher overall DA content in the striatum. In addition, the apparent Tc was significantly faster in the striatum than in the SN, consistent with the higher density of DA transporter (DAT) sites within the former region. The red/ox ratio of signals observed within the SN and striatum was 1.45 ± 0.05 and 1.17 ± 0.02, respectively. These ratios are consistent with the release of DA, rather than 5-HT, which is reportedly released in the rat SN (Rice et al., 1994; Ivavani and Kruk, 1997).

**Reproducibility of KCl-Evoked Response**

To determine whether single applications of 30 mM KCl might have detrimental effects on the viability of the slices, a series of experiments were performed in which successive applications of KCl were performed at 30-min intervals. As shown in Fig. 1, superfusion of 30 mM KCl produced reproducible responses in both the striatum and SN. The ratio of the peak amplitude of the first response to the peak amplitude of the second response, designated S2/S1, was 0.87 ± 0.09 for the striatum and SN, respectively (n = 10 slices from each brain region).

**Modulation of KCl-Evoked Responses by Pharmacological Treatments**

In terms of both the amplitudes and red/ox ratios of the observed responses, the electrochemical signals seen after KCl application were consistent with DA release in both the striatum and SN. Moreover, the reproducibility of these responses minimized the possibility that such release was non-specific, due to the death of cells after depolarization. To further compare the properties of the KCl-evoked release of DA, several pharmacological treatments were performed between successive stimulations within the same slice. A summary of the effects of all drug treatments on the S2/S1 ratios in each brain region is shown in Table 2.

**Effects of TTX.** Blockade of voltage-gated sodium channels with TTX has been previously used to establish release mechanisms in both nerve terminals and cell soma (Santiago and Westerink, 1990; Heering and Ahercome, 1995). We compared the sensitivity of the observed KCl-evoked responses to TTX in both the SN (n = 7) and striatum (n = 9) by applying TTX (0.5 μM) between the first and second KCl stimuli. Representative experiments are shown in Figs. 2A

**TABLE 1**

Summary of KCl-evoked electrochemical signals in brain slices

<table>
<thead>
<tr>
<th></th>
<th>Striatum (n = 84)</th>
<th>SN (n = 80)</th>
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<tr>
<td>Amplitude</td>
<td>μM</td>
<td>μM/s</td>
</tr>
<tr>
<td>S1</td>
<td>4.40 ± 0.37*</td>
<td>0.061 ± 0.006*</td>
</tr>
<tr>
<td>S2</td>
<td>0.38 ± 0.02</td>
<td>0.003 ± 0.001</td>
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</table>

* p < 0.05, Student’s t test
TABLE 2
Summary of drug effects on KCl-evoked DA release
Data are expressed as mean ± S.E.M. of the S2/S1 ratio. Comparisons were performed using a two-way ANOVA (treatment × brain region) followed by Tukey's post hoc comparisons. Drugs were applied to bath at indicated concentrations between the first (S1) and second (S2) KCl stimuli. Sulpiride significantly potentiated S2/S1 ratio in striatum (*p < .05 from control), and TTX and CdCl2 significantly reduced the S2/S1 ratio in striatum (*p < .05 from control). In nigra, TTX significantly (**p < .05) reduced the S2/S1 ratio from control. However, sulpiride and CdCl2 alone did not affect S2/S1 ratio (**p < .05 versus effect in striatum). Cocaine did not affect S2/S1 ratios in either brain region.

<table>
<thead>
<tr>
<th></th>
<th>Striatum</th>
<th>SN</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>0.87 ± 0.11</td>
<td>0.81 ± 0.09</td>
</tr>
<tr>
<td>TTX (0.5 μM)</td>
<td>0.11 ± 0.05*</td>
<td>0.31 ± 0.04*</td>
</tr>
<tr>
<td>CdCl2 (500 μM)</td>
<td>0.04 ± 0.01*</td>
<td>0.73 ± 0.05**</td>
</tr>
<tr>
<td>TTX + CdCl2</td>
<td>N.D.</td>
<td>0.21 ± 0.03*</td>
</tr>
<tr>
<td>(±)-Sulpiride (50 μM)</td>
<td>1.31 ± 0.07*</td>
<td>0.67 ± 0.16**</td>
</tr>
<tr>
<td>Cocaine (50 μM)</td>
<td>0.98 ± 0.12</td>
<td>0.73 ± 0.11</td>
</tr>
</tbody>
</table>

N.D., not determined.

(striatum) and 3A (SN). In both brain regions, the application of TTX resulted in a significant decrease in the S2/S1 ratio from control values. The reduction in S2/S1 ratios was not significantly different between the SN and striatum (two-way ANOVA, drug × region, p > .05).

**Effects of Cadmium.** The observation that TTX attenuated, but did not completely eliminate, release in the SN and striatum could be explained by the direct activation of voltage-sensitive Ca2+ channels. To address this issue, slices were superfused with cadmium chloride (CdCl2; 500 μM), a nonselective blocker of these channels. As shown in Fig. 2B, in the striatum (n = 8), CdCl2 application significantly reduced the S2/S1 ratio from the control value. In contrast, superfusion of SN slices (n = 7) with CdCl2 did not significantly affect the S2/S1 ratio (Fig. 3B; p > .05 compared with control). Coapplication of TTX and cadmium in SN slices (n = 7) was no more effective in reducing the S2/S1 ratio than TTX alone.

**Removal of Ca2+.** The lack of an effect of cadmium on the release process within the SN would imply that calcium influx through voltage-sensitive Ca2+ channels is not an obligatory step in somatodendritic DA release. To test the general dependence of release on extracellular Ca2+ levels, slices were superfused with a Ca2+-free buffer. The amplitude of the KCI-evoked response in this buffer was then compared with a subsequent KCI-evoked response when CaCl2 (1 mM) was added back to the superfusion chamber. Representative experiments for both the striatum and SN are shown in Figs. 2C and 3C, respectively. KCI-evoked release of DA in the striatum (n = 8) was greatly diminished in the Ca2+-free buffer but could be enhanced by subsequent addition of calcium to the bath. In contrast, in SN slices (n = 9), release was nearly identical in the absence or presence of external Ca2+. A summary of the effects of Ca2+ removal on DA release is shown in Fig. 4.

**Effects of Cocaine.** The TTX dependence of DA release observed in the SN implies that a voltage-sensitive process resulting in DA release occurs in response to KCl depolarization. Given the recent findings that the DAT is regulated in a voltage-dependent fashion (Sonders et al., 1997), as well as a recent study that showed veratridine-induced DA release can be blocked by DAT inhibition (Elverfors et al., 1997), we investigated whether there might be a DAT-mediated component of the KCI-evoked DA release in the SN. As shown in Table 2, superfusion of cocaine (50 μM) between successive KCl applications did not significantly affect the S2/S1 ratios in either the striatum or SN (n = 10 signals per region).

**Effects of D2 Antagonism on Release.** As the experiments described above demonstrate, there are fundamental differences in DA-release properties between the striatum and SN. Another regulator of DA release is the D2 autoreceptor, the activation of which has been shown to reduce DA release (Nissbrandt et al., 1989; Cragg and Greenfield, 1997). To determine whether the KCI-evoked DA release in both the striatum and SN was subject to regulation by the D2 receptor, slices were superfused with the D2 receptor antagonist (±)-sulpiride (50 μM) between successive stimulations. Representative data are shown in Fig. 5. As shown in Table 2, application of sulpiride significantly increased the S2/S1 ra-

![Fig. 2. Effects of various pharmacological manipulations on the KCl-evoked electrochemical responses in striatal slices. In all cases, the solid black bars indicate the 30 mM KCl stimulus. A, TTX (0.5 μM) was applied between stimuli (hatched bar). B, 500 μM CdCl2 was applied between stimuli. C, slices were incubated in and perfused with Ca2+-free buffer during the first stimulus, and CaCl2 (1 mM, gray bar) was added back before the second stimulus.](image)
ratio in striatal slices (n = 7), consistent with the modulatory capability of terminal D2 receptors. In contrast, the S2/S1 ratio was not significantly altered by sulpiride application in the SN (n = 7), suggesting that somatodendritic release of DA is not subject to D2 autoreceptor-mediated control under these release conditions.

Effects of d-Amphetamine on DA Release

Another drug that is known to promote DA release is d-amphetamine, which acts through the DAT to promote efflux of DA into the extracellular space. Because this agent acts in a Ca\(^{2+}\)-independent fashion and given the observed differences in calcium dependence of the KCl-evoked DA release between the SN and striatum, we compared d-amphetamine-evoked DA efflux with KCl-evoked responses. As shown in Fig. 6, DA release evoked by d-amphetamine superfusion in striatal slices was dose dependent and could be partially inhibited by coapplication of the DAT inhibitor cocaine. Only the highest dose (500 \(\mu M\)) of d-amphetamine was used in the SN. However, we also considered the possibility that at this higher concentration, some 5-HT release might

Fig. 3. Effects of various pharmacological manipulations on the KCl-evoked electrochemical responses in slices containing the SN. The solid black bars indicate the 30 mM KCl stimulus. A, TTX (0.5 \(\mu M\)) was applied between stimuli (hatched bar). B, CdCl\(_2\) (500 \(\mu M\)) was applied (hatched bar). C, slices were first prepared in and superfused with a Ca\(^{2+}\)-free buffer during the first stimulus, and CaCl\(_2\) (1 mM, gray bar) was added back to the medium before the second KCl application. Note that in contrast to striatal signals, electrochemical responses in the SN appear to be relatively unaffected by either CdCl\(_2\) application or Ca\(^{2+}\) removal.

Fig. 4. Summary of the effects of Ca\(^{2+}\) removal on KCl-evoked release in the striatum (n = 8) and substantia nigra (n = 9). Release in the Ca\(^{2+}\)-free condition is represented as a percentage of release observed when 1 mM Ca\(^{2+}\) was added back to the medium. There was a significant (***, p < .001, Student’s two-tailed t test) difference between release under these conditions in the striatum compared with the SN.

Fig. 5. Effect of D2 antagonism on the KCl-evoked electrochemical responses in the striatum (top) and SN (bottom). (±)-Sulpiride (50 \(\mu M\)) was applied for the time indicated (striped bar) between the first and second KCl applications (solid bars). Note the enhancement of the response in the presence of sulpiride in the striatum, whereas the signal is slightly diminished in the SN after sulpiride application.
also be detected in the SN (Cragg et al., 1997b; Iravani and Kruk, 1997). Therefore, we performed superfusion experiments in nigral slices in which varying mixtures of DA and/or 5-HT were applied to the tissue. As shown in Fig. 7, DA and 5-HT were clearly distinguishable based on their individual red/ox ratios. As the 5-HT/DA ratio was increased, the red/ox ratios were seen to decrease. However, even at a 5x greater 5-HT concentration, the signals were still observed to have higher (e.g., DA-like), rather than lower (5-HT like), ratios. Thus, for the endogenous release evoked by \textit{d}-amphetamine, all signals with red/ox ratios $>1$ were considered to be predominantly DA like, with little contribution from 5-HT. A summary of electrochemical responses evoked by \textit{d}-amphetamine application is given in Table 3.

Figure 8 shows a comparison of representative KCl- and \textit{d}-amphetamine-evoked responses in the two brain regions. Although the onset and decay of the response were slower in both the SN and striatum, consistent with the mechanism of action of \textit{d}-amphetamine, the peak amplitude of the response was nearly identical within the SN, regardless of the agent used. In contrast, release evoked by KCl application was consistently higher in the striatum than that evoked by \textit{d}-amphetamine.

### Table 3

<table>
<thead>
<tr>
<th></th>
<th>Striatum ($n = 20$)</th>
<th>Substantia nigra</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Amplitude (\mu M)</strong></td>
<td>1.14 ± 0.17</td>
<td>0.36 ± 0.09</td>
</tr>
<tr>
<td><strong>Red/Ox</strong></td>
<td>1.24 ± 0.05</td>
<td>1.01 ± 0.09</td>
</tr>
<tr>
<td><strong>Tc (\mu M/s)</strong></td>
<td>0.0050 ± 0.003</td>
<td>0.0004 ± 0.0001*</td>
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Fig. 6. Dose-dependent effects of \textit{d}-amphetamine on DA release in striatal slices. Concentrations of 50 \mu M ($n = 11$), 100 \mu M ($n = 30$), or 500 \mu M ($n = 20$) were bath applied in individual striatal slices. When cocaine (50 \mu M) was applied 10 min before and during the application of \textit{d}-amphetamine ($n = 5$ slices), the electrochemical response was attenuated (*$p < .05$, two-tailed Student's $t$ test), demonstrating the role of DAT in mediating the response.

Fig. 7. Summary of the observed in situ red/ox ratios in response to bath superfusion of varying mixtures of DA and 5-HT. Multiple ($n = 6$ different electrodes) recordings were performed in SN slices and DA or 5-HT was applied at varying concentrations (10–50 \mu M) to the bath to produce detectable signals at the electrodes (generally <1 \mu M). DA alone (open bar) yielded the highest red/ox ratios, whereas 5-HT alone (solid bar) yielded the lowest. When the 5-HT/DA ratio was systematically increased, the red/ox ratio decreased, with significant effects (*$p < .05$ versus DA alone, one-way repeated measures ANOVA followed by Tukey’s post hoc comparisons) observed at 2:1 and 5:1. Red/ox ratios in response to equal (1:1) concentrations of DA and 5-HT were not significantly different than red/ox ratios for DA alone.

Fig. 8. Comparison of the amplitude and time course of the KCl- (30 mM) and \textit{d}-amphetamine- (\textit{d}-Amp, 500 \mu M) evoked electrochemical responses in the striatum (top) and SN (bottom). Data are representative of signals typically observed in response to these agents. Note that \textit{d}-Amp promotes a much slower release profile in both brain regions, consistent with its complex mechanism of action. However, the peak amplitude of the response to both KCl and \textit{d}-amphetamine is similar in the SN but disparate in the striatum.
Discussion

In the present study, two chemical stimuli, KCl and d-amphetamine, were used to compare DA release in the striatum and SN. We report several findings that highlight differences in somatodendritic release versus axonal release of DA. First, the magnitude of the response to KCl is, on average, 11× higher in the striatum than in the SN. Second, although KCl-evoked release of DA is both TTX and Ca2+ sensitive in the striatum, it appears to be TTX sensitive but Ca2+ insensitive in the SN. Third, although DA release in the striatum can be enhanced by application of the D2 antagonist sulpiride, similar modulation is not observed in the SN. Finally, we report that although d-amphetamine and KCl produce DA-like electrochemical responses of similar magnitude in the SN, KCl-evoked responses are consistently larger in the striatum, relative to those produced by d-amphetamine.

Several techniques have been used to study the properties of somatodendritic DA release, including in vivo microdialysis, slice superfusion, and voltammetric recordings (Heeringa and Abercrombie, 1995; Elverfors et al., 1997; Rice et al., 1997). Of these approaches, voltammetry is often preferred due to its excellent sensitivity as well as spatial and temporal resolution (Kawagoe et al., 1993). In the present study, chronoamperometric recordings were used to measure DA release in response to chemical stimuli. We used chemical stimulation, rather than the electrical stimulation protocol used by other investigators, for several reasons. First, the electrical stimulation conditions required to produce DA release involve the application of a train of high-frequency (10–100 Hz), high-voltage pulses (18–20 V). It is striking to note that under these conditions, even DA release in the striatum has been reported to be TTX insensitive (Rice et al., 1997). In contrast, we found that KCl application produces striatal DA release that is both TTX and Ca2+ sensitive, which is consistent with previous reports (Osborne et al., 1990; Santiago and Westerink, 1991a; Jacob and Cox, 1992; Bergquist et al., 1998). Second, the electrical stimulation protocol tends to irreversibly deplete releasable DA pools (Rice et al., 1997), whereas the KCl application protocol reported here is highly reproducible. This allows for pharmacological manipulations to be performed between successive stimuli rather than having to move the electrode to an alternate location, where DA release may vary depending on the number of available release sites. Third, we note that under conditions of KCl application, the electrochemical signals observed in both the striatum and SN are likely to be due almost exclusively to the release of DA, as demonstrated by red/ox ratios of the signals (Hoffman et al., 1998). In contrast, using electrical stimulation, others have reported that in rat brain slices, 5-HT signals predominate over DA signals in the SN (Rice et al., 1994; Cragg et al., 1997a). As we have described here, DA and 5-HT can be distinguished in situ based on their red/ox ratios, and the red/ox ratio of DA is only reduced when large concentrations of 5-HT are present (see Fig. 7). Indeed, depending on the stimulus used to promote release (KCl versus d-amphetamine in the present study), it is possible to observe signals that reflect predominantly DA release, as well as mixtures of DA and 5-HT. Thus, DA release can routinely be observed using the chemical stimulation protocol described here.

Several investigations into the mechanisms of DA release within the SN have led to the suggestion that the somatodendritic release of DA differs from the release observed at striatal synapses. Early studies by Groves and Linder (1983) suggested that much of the DA stored within the SN is not contained within classic synaptic vesicles. More recently, ultrastructural analysis has revealed that within the SN, the vesicular monoamine transporter-2 (VMAT-2) is associated more with the endoplasmic reticulum than with dense core synaptic vesicles (Nirenberg et al., 1996). These data, coupled with a lack of synaptic specializations within the SN, suggest that somatodendritic DA release does not rely on “classic” synaptic release mechanisms. Although most evidence supports a TTX-sensitive component of somatodendritic release (Santiago and Westerink, 1990; Robertson et al., 1991; Heeringa and Abercrombie, 1995), there is at least one recent report that suggests that release may be TTX insensitive (Rice et al., 1997). We report that KCl-evoked DA release within the SN is TTX sensitive but does not appear to require the activation of voltage-sensitive Ca2+ channels, as demonstrated by the failure of CdCl2 to attenuate the electrochemical signals. The calcium independence of the release process in the SN was further revealed by experiments involving the removal of Ca2+ from the buffer, which attenuated DA release in the striatum but not in the SN. Although Elverfors et al. (1997) recently demonstrated Ca2+-dependent DA release in the SN, the methodological approaches used were vastly different from those used here. The TTX-sensitive, Ca2+-insensitive release of DA that we report was unexpected because it is assumed that the depolarization mediated by activation of voltage-sensitive sodium channels would activate voltage-sensitive Ca2+ channels, thereby promoting transmitter release (Rice et al., 1997). Indeed, Ca2+ channel activation appears to be the mechanism of release observed in the striatum in the present study. Our data do not discount a role for Ca2+ channels in modifying the electrophysiological properties of DA neurons, which have been well demonstrated (Nedergaard et al., 1988; Kang and Kitai, 1993). However, in the SN, it appears as though activation of sodium channels serves to activate some other voltage-sensitive element or elements to promote DA release. In this regard, it is interesting to note that veratradine-evoked DA release in the SN also occurs through a Ca2+-independent mechanism that was shown to be due to DAT reversal (Elverfors et al., 1997). Although there is evidence that the DAT may function in a voltage-dependent fashion (Sonders et al., 1997), it appears that DAT reversal is not responsible for the KCl-evoked release because cocaine did not inhibit DA release. We cannot exclude the possibility that Ca2+ influx through other sources may play a role in somatodendritic release, although the experiments involving removal of extracellular Ca2+ make this unlikely. It is also possible that KCl-induced depolarization mobilizes intracellular Ca2+ stores that facilitate DA release. Further studies will be required to establish the precise mechanism or mechanisms through which this release occurs. However, based on these studies, we propose that KCl-evoked release in the SN is a voltage-dependent, but not necessarily a Ca2+-dependent, process.

The amount of DA released during nerve stimulation reflects a combination of both release and reuptake processes. For the latter, the DAT is the most critical element, both in the striatum and, as we and others have recently demon-
strated, within the SN (Cragg et al., 1997b; Hoffman and Gerhardt, 1998; Hoffman et al., 1998). In terms of controlling DA release, the D₂ autoreceptor represents an important regulatory mechanism in that activation of these receptors inhibits further DA release (Santiago and Westerink, 1991b; Cragg and Greenfield, 1997). The D₂ receptor is localized both on tyrosine hydroxylase-containing axons in the striatum and on dendrites within the SN (Sesack et al., 1994). Given the differences in release properties that we observed between the SN and striatum, we investigated whether D₂ regulation of DA release differed in these regions. We report that KCl-evoked DA release is enhanced by application of the D₂ antagonist sulpiride in the striatum, as evidenced by the increase in the S2/S1 ratio. In contrast, no significant effect of sulpiride on DA release was seen in the SN. The differences in autoreceptor-mediated control of DA release that we report here are similar to those reported by others who have found that D₂-mediated inhibition of DA release is less pronounced in the SN than in the striatum (Nissbrandt and Hjorth, 1992; Cragg and Greenfield, 1997). Together with the differences we observe in the Ca²⁺ dependence of release within the SN compared with the striatum, the differences in autoreceptor control again suggest that DA-release properties differ greatly between somatodendritic and terminal elements.

If KCl-evoked DA release within the SN is governed by a predominantly Ca²⁺-independent process, then one would predict that an agent that acts through a Ca²⁺-independent mechanism should be equally efficacious as KCl in promoting DA release. Because d-amphetamine acts in a Ca²⁺-independent manner to promote DA efflux through reversal of DAT (Carboni et al., 1989; Westerink et al., 1989; Giro et al., 1996), we compared the efficacy of this drug in promoting DA release in the SN and striatum. Bath applications of d-amphetamine produced a dose-dependent increase in the electrochemical signal amplitude in the striatum. Even at the highest dose (500 μM) used, however, the mean signal amplitudes still only achieved ~25% of the signal produced by KCl application. In contrast, when d-amphetamine was applied in the SN, the DA signals (e.g., those with DA-like red/ox ratios) achieved approximately the same amplitude as those produced by KCl. As with KCl, the mean amplitude of the d-amphetamine-evoked signals was higher in the striatum than in the SN, as predicted by differences in whole tissue DA levels (Hebert and Gerhardt, 1997). However, although differences in KCl-evoked DA release were 11-fold greater in the striatum than the SN, these differences were less pronounced (3- to 4-fold) when d-amphetamine was used to promote release. The differences in Ca²⁺ sensitivity of KCl-evoked release of DA between these two brain regions, coupled with regional differences between the magnitude of KCl- and d-amphetamine-evoked DA release, imply that the balance between Ca²⁺-dependent and Ca²⁺-independent release processes are markedly different between the striatum and SN.

In summary, using high-speed chronoamperometric recordings in brain slices, we found that somatodendritic release of DA within the SN differs from striatal DA release in at least two major ways. First, DA release does not appear to be modulated through the activation of the D₂ receptor in the SN, as it does in the striatum. Second, although DA release in both regions is sensitive to TTX, release within the SN apparently is not dependent on Ca²⁺ influx, as demonstrated by the lack of effects of cadmium and calcium removal. This finding, coupled with the similar magnitude of the KCl- and d-amphetamine-evoked release in the SN, suggests that Ca²⁺-independent DA release may play a greater role within the SN compared with that in the striatum.

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Jacocks HM3 and Cox BM (1992) Serotonin-stimulated release of [3H]dopamine via a Ca²⁺-independent process, then one would predict that an agent that acts through a Ca²⁺-independent mechanism should be equally efficacious as KCl in promoting DA release. Because d-amphetamine acts in a Ca²⁺-independent manner to promote DA efflux through reversal of DAT (Carboni et al., 1989; Westerink et al., 1989; Giro et al., 1996), we compared the efficacy of this drug in promoting DA release in the SN and striatum. Bath applications of d-amphetamine produced a dose-dependent increase in the electrochemical signal amplitude in the striatum. Even at the highest dose (500 μM) used, however, the mean signal amplitudes still only achieved ~25% of the signal produced by KCl application. In contrast, when d-amphetamine was applied in the SN, the DA signals (e.g., those with DA-like red/ox ratios) achieved approximately the same amplitude as those produced by KCl. As with KCl, the mean amplitude of the d-amphetamine-evoked signals was higher in the striatum than in the SN, as predicted by differences in whole tissue DA levels (Hebert and Gerhardt, 1997). However, although differences in KCl-evoked DA release were 11-fold greater in the striatum than the SN, these differences were less pronounced (3- to 4-fold) when d-amphetamine was used to promote release. The differences in Ca²⁺ sensitivity of KCl-evoked release of DA between these two brain regions, coupled with regional differences between the magnitude of KCl- and d-amphetamine-evoked DA release, imply that the balance between Ca²⁺-dependent and Ca²⁺-independent release processes are markedly different between the striatum and SN.

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