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In vivo interaction of cocaine with the dopamine transporter as measured by voltammetry

Phillip G. Greco, Paul A. Garris *

Cellular and Integrative Physiology Section, Department of Biological Sciences, Illinois State University, 210 Julian Hall, Normal, IL 61790-4120, USA

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Abstract

The goal of this review is to describe what the voltammetry technique tells us about cocaine–dopamine transporter (DAT) interactions and the subsequent changes in extracellular dopamine levels in the brain. The primary advantage of voltammetry, in this regard, is the capability for kinetic analysis in situ. Analysis of electrically evoked dynamics suggests that cocaine competitively inhibits dopamine uptake in the caudate-putamen and nucleus accumbens with a similar efficacy. The preferential increase in accumbal dopamine following systemic cocaine administration was found to be related not to a unique cocaine–DAT interaction, but rather to a unique combination of dopamine release and uptake rates. Similar enhancement occurs in sub-regions of the caudate-putamen exhibiting this release and uptake combination. Other factors such as diffusion and whether dopaminergic signaling is tonic or phasic also determine the effects of cocaine on striatal dopamine levels.

Keywords: Cocaine; Dopamine; DAT (dopamine transporter); Release; Diffusion; Voltammetry

1. Introduction

Important behavioral effects of cocaine are mediated at the cellular level in the brain by the interaction of this drug with the dopamine transporter (DAT) and the subsequent change in extracellular dopamine concentration (Koob and Bloom, 1988; Kuhar et al., 1991; Giros et al., 1996). As an uptake inhibitor, cocaine interferes with DAT function. Slowing the clearance rate of released dopamine enhances the action of the neurotransmitter by increasing its concentration, lifetime and diffusion in extracellular fluid (Carboni et al., 1989; Kuczenski and Segal, 1992; Cass et al., 1992; Jones et al., 1995a). Several approaches have been used to assess cocaine–DAT interactions including [^{3}H]dopamine uptake (Izenwasser et al., 1990), ^{3}H-cocaine binding (Boja and Kuhar, 1989), combined uptake and binding studies (Reith et al., 1998), DAT expression in cell lines (Eshleman et al., 1999), site directed mutagenesis of DAT (Lin and Uhl, 2002), comparison to chemical analogues (Ghorai et al., 2003), and various voltammetric techniques (Schenk et al., 1990; Zahniser et al., 1999; Wu et al., 2001a).

In the next two sections of this review, we focus on the information that voltammetry provides about dopamine uptake and cocaine–DAT interactions. Voltammetry affords the unique opportunity to assess cocaine–DAT interactions in the intact brain. Indeed, the temporally resolved measurements are suitable for monitoring the rapid clearance of dopamine from extracellular space and kinetic analysis. The small size of the voltammetric microsensor, moreover, can detect differences in dopamine uptake on a microscopic scale. An additional advantage to the study of cocaine is that voltammetry characterizes release, uptake and diffusion, the primary mechanisms regulating extracellular dopamine in the brain. How these mechanisms play a critical role in determining the magnitude of cocaine-induced dopamine increases is also discussed in this review.

2. Voltammetry coupled to electrical stimulation, a tool for studying uptake kinetics

The classic approach for assessing cocaine–DAT interactions is [^{3}H]dopamine uptake into synaptosomes (Horn et al., 1971). After correcting for nonspecific uptake, a plot of

* Corresponding author. Tel.: +1-309-438-2664; fax: +1-309-438-3722.
E-mail address: pagarri@ilstu.edu (P.A. Garris).

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transport velocity versus substrate concentration yields the rectangular hyperbola of Michaelis–Menten kinetics. K_m, inversely related to the affinity of DAT for dopamine, and V_{max}, related to the total number and turnover of DAT, are readily determined through various linear transformations. Using the classical approach, unfortunately, cocaine has been found to act as a competitive inhibitor by altering K_m (Cao et al., 1989; Krueger, 1990), a noncompetitive inhibitor by altering V_{max} (Missale et al., 1985), or an uncompetitive inhibitor by altering both K_m and V_{max} (Wheeler et al., 1994).

The origin of this discrepancy is not established but could be related to different in vitro conditions (Povlock et al., 1996). Having the animal establish conditions for uptake is advantageous, but kinetic evaluation is difficult in intact tissue. Voltammetric microsensors coupled to electrical stimulation overcome these difficulties to provide a means for the study of cocaine–DAT interactions in vivo. As will be shown in the fourth section, this approach is also well suited for assessing what factors are important for determining cocaine effects on extracellular dopamine.

The voltammetry experiment is straight forward (Fig. 1A): a carbon-fiber microelectrode is implanted in a dopaminergic projection field and a stimulating electrode is positioned near the locus of dopaminergic cell bodies or ascending dopaminergic fibers. One important point to consider is that only when the stimulating electrode (Fig. 1B) and voltammetric microsensor (Fig. 1C) are positioned near dopaminergic neurons is dopamine release observed. Results indicating that the dopamine interacting with DAT originates from dopaminergic neurons are not trivial. The use of an exogenous dopamine source, e.g., pressure ejection or iontophoresis (Gerhardt and Palmer, 1987), suggests the possibility that, within the spatial resolution of the microsensor, dopamine uptake is assessed in a region where the neurotransmitter may not normally be released.

Uptake information is contained within the voltammetric recording (Fig. 2). As simulated in Panel A, transient electrical stimulation at a high frequency results in a spike of extracellular dopamine. Release and uptake combine to determine the increase in extracellular dopamine during the pulse train. Afterwards, only uptake operates to return dopamine to pre-stimulus levels. The post-stimulus clearance curve (Fig. 2B) is readily converted to the Michaelis–Menten hyperbola by taking the first derivative (i.e., instantaneous velocity) and plotting it versus dopamine concentration (Fig. 2C). V_{max}, the asymptote of the hyperbola, is the linear portion of the clearance curve at high concentrations. K_m, the substrate concentration at one half of V_{max} on the hyperbola, is found near the inflection point where uptake velocity changes the greatest. Consequently, the second derivative plotted versus time yields a peak near K_m (Fig. 2D).

The simulations described in Fig. 2 demonstrate that, in theory, electrically evoked dopamine dynamics measured by

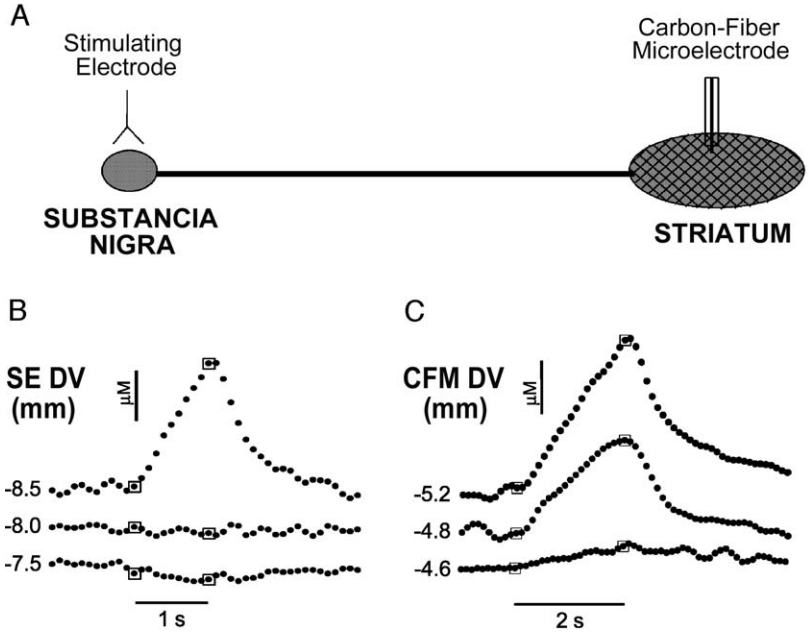

Fig. 1. Fast-scan cyclic voltammetry coupled with electrical stimulation. (A) A bipolar stimulating electrode was lowered to dopaminergic cell bodies in the substantia nigra. Following stimulation, a carbon-fiber microelectrode recorded dopamine release in the dopaminergic projection fields of the striatum. (B) Dopamine release was only observed when the stimulating electrode was lowered into the substantia nigra. The carbon-fiber microelectrode was held at a fixed position. Electrical stimulation consisted of a 60-Hz train of pulses applied for 1 s. (C) Dopamine release was heterogeneous in the striatum. The position of the stimulating electrode was fixed during application of the 2 s, 60-Hz pulse train. Time and concentration scales are different in B and C. Abbreviations: SE, stimulating electrode; CFM, carbon-fiber microelectrode; DV, dorsoventral.

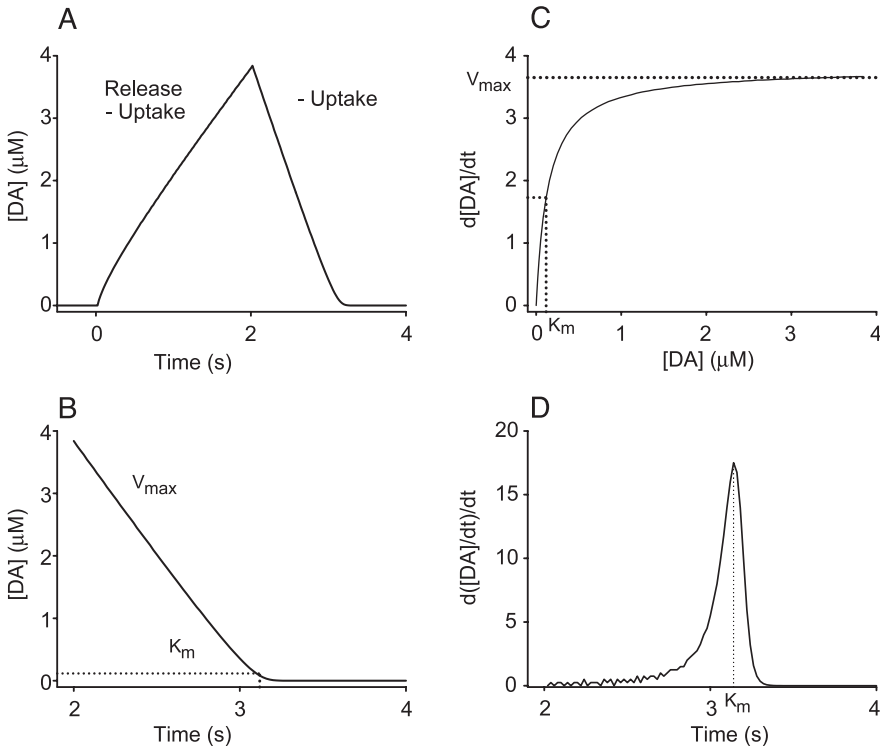

Fig. 2. 'Classic' analysis of dopamine uptake. (A) Simulated response to electrical stimulation by a 60-Hz, 2-s pulse train. (B) Clearance portion of the electrically evoked response. (C) First derivative of the clearance curve plotted against concentration. (D) Second derivative of the clearance curve plotted against time.

voltammetry are analyzed identically to classic uptake experiments. In fact, voltammetry potentially offers advantages. Because a semi-continuous record of uptake is provided, the entire rectangular hyperbola is collected in one sample assuming sufficiently high dopamine concentrations ($[DA] \gg K_m$) are elicited. The semi-continuous trace also affords the unique calculation of a second derivative to identify K_m. Unfortunately, practice suggests that such analysis is not possible. The primary problem is calculation of K_m, which at 0.2 μM (Horn, 1990) approaches the detection limit for voltammetry (~ 0.1 μM). The microsensor also does not provide a "pure" kinetic measure of dopamine uptake at the end of the clearance curve where K_m resides because of diffusional distortion (Wu et al., 2001b), adsorption and desorption of dopamine at the carbon fiber (Bath et al., 2000; Venton et al., 2002), and interference from extracellular ions (Rice and Nicholson, 1995; Jones et al., 1995b). Calculating K_m from a single point or exclusively from the tail end of the clearance curve is therefore tenuous with voltammetry.

To overcome limitations of voltammetry for classic uptake analysis, we have devised an alternative strategy for assessing cocaine–DAT interactions (Wu et al., 2001a). The cornerstone is quantitative kinetic analysis of not only the entire electrically evoked signal, but also of a series of signals evoked by different frequencies. These evoked measurements are curve fit to a neurochemical model describing the rising phase rate as a balance between the opposing actions of release and uptake (Wightman et al., 1988):

$$d[DA]dt = [DA]_p * f - V_{max}/([DA]/K_m) + 1 \quad (1)$$

where $[DA]_p$ is the release term and defined as the concentration of dopamine elicited per stimulus pulse, and f is the frequency of the stimulation. The second half of the expression is the Michaelis–Menten equation. The uptake expression solely describes the falling phase of the evoked signal. The reason the entire evoked signal is analyzed is that uptake operates throughout the measured response. Thus, the "cleanest" portion of the voltammetric signal, i.e., the earliest measurements in time, is also used to determine dopamine uptake parameters.

The use of several frequencies enhances the rigor of the kinetic analysis by providing signals for curve fitting with different dynamics (Fig. 3A). The determining factor for the different dynamics is not differential rates for dopamine uptake and release, which are frequency independent, but rather time for uptake in between stimulus pulses. At lower frequencies (e.g., 10 Hz), ample time allows uptake to compete with release and establish a steady-state level where these rates equal. Time is limited at higher frequencies (e.g., 60 Hz) such that release overwhelms uptake resulting in a spike of extracellular dopamine. The responses evoked by middle frequencies (e.g., 30 Hz) exhibit a mixture of steady-state and peak-shaped dynamics. Curve

fitting is robust, because the frequency series is analyzed simultaneously to determine one set of release ($[DA]_p$) and uptake (K_m and V_{max}) parameters.

Regardless of how powerful the curve fitting algorithm is, spurious fits are a concern with the less than ideal voltammetric measurements. To address this issue, our strategy to assess cocaine–DAT interactions involves additional semi-quantitative and qualitative components. For example, Eq. (1) is algebraically re-arranged in different forms to calculate the three parameters, $[DA]_p$, K_m and V_{max}, individually. This semi-quantitative approach, called single curve analysis (Wu et al., 2001b), requires assumptions not needed for curve fitting and therefore may not be applicable to all measurements. Mechanism is revealed qualitatively by drug effects on the time-dependent changes of extracellular dopamine (Fig. 3B). Distinguishing competitive and non-competitive uptake inhibitors is unambiguous due to the remarkable slowing of the clearance rate by a reduction in V_{max}. Increasing K_m causes a considerably less prominent effect. In fact, these simulated curves are largely indistinguishable from those calculated from an increase in $[DA]_p$. One subtle exception is that the inflection point of the clearance curve is extended out and up for an increase in K_m.

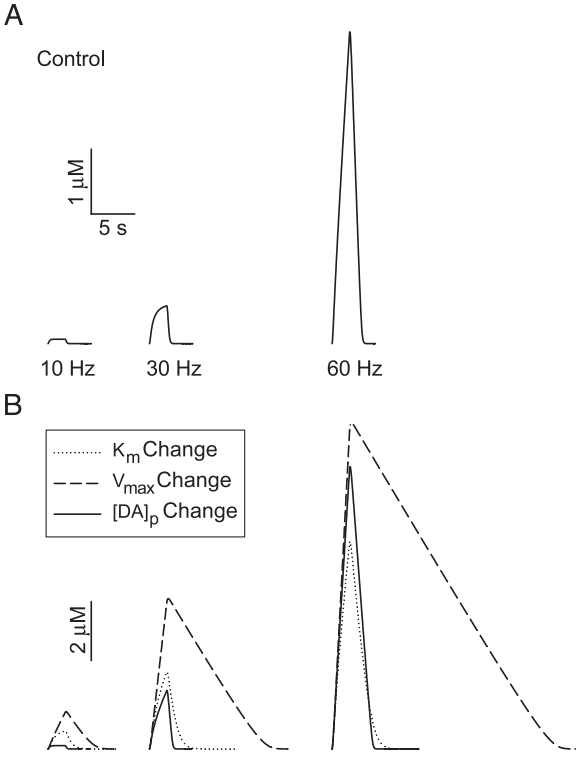

Fig. 3. (A) Simulated responses for electrically evoked dopamine transients. (A) Control curves. Parameters were: $[DA]_p = 0.1$ µM; $K_m = 0.2$ µM; $V_{max} = 4$ µM/s. (B) Curves calculated with altered parameters for release and uptake. Values for altered $[DA]_p$, K_m and V_{max} were 0.143 µM, 2.0 µM, and 0.5 µM/s, respectively. Only one parameter was changed for each set of curves. The frequency indicated under Panel A also applies to Panel B. Parameters for dopamine release and uptake are based on the study of Wu et al. (2001a, 2002).

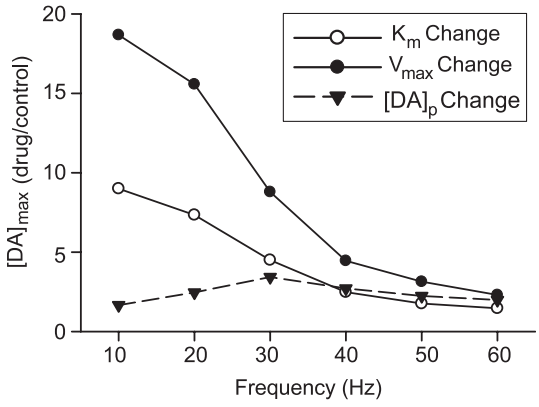

Fig. 4. Simulated frequency series. Curves were simulated using the release and uptake parameters described in the legend for Fig. 3. $[DA]_{max}$ (drug/control) for the *y*-axis was obtained by dividing the response with the altered mechanism by the control response.

Another qualitative means for identifying mechanism is the relationship between the drug-induced increase in $[DA]_{max}$, the maximal dopamine concentration elicited, and frequency (Fig. 4). Releasers and uptake inhibitors exhibit quite different profiles and are thus readily distinguished. Whereas releasers elicit the greatest effects at middle frequencies (e.g., 30 Hz) resulting in a peak-shaped frequency dependence, uptake inhibitors act preferentially at lower frequencies. Discriminating competitive from non-competitive uptake inhibitors is more difficult but not impossible, because the latter exhibits a steeper relationship between $[DA]_{max}$ and frequency. Effects of a competitive inhibitor at lower frequencies may also level off (Wu et al., 2001a). One important point to emphasize is that all three conditions, a change in $[DA]_p$, K_m or V_{max}, increase $[DA]_{max}$ at each frequency, indicating that this general effect is a poor index of drug mechanism.

3. Cocaine, a competitive inhibitor of DAT in vivo

We have used the strategy described in the previous section to evaluate cocaine–DAT interactions in vivo (Wu et al., 2001a). In this experiment, fast-scan cyclic voltammetry (FSCV) at carbon-fiber microelectrodes was used to monitor electrically evoked dopamine levels in the striatum of the anesthetized rat. By virtue of scanning the potential of the carbon fiber, FSCV provides information in the form of a voltammogram to identify the analyte detected (Michael et al., 1998). Microsensors were implanted in the caudate-putamen and nucleus accumbens for simultaneous recording in both striatal regions, and a stimulating electrode was positioned in the medial forebrain bundle. To aid in the assessment of mechanism, cocaine was compared to an established competitive inhibitor, nomifensine (Gianutsos et al., 1982; Tuomisto, 1977), and a new, irreversible (i.e.,

noncompetitive) inhibitor, RTI-76 (Wang et al., 2000; Fleckenstein et al., 1996). Drug effect was compared to saline-injected controls.

Inspection of electrically evoked dynamics demonstrated that RTI-76, but not cocaine or nomifensine, markedly slowed the clearance rate of released dopamine (Wu et al., 2001a). This result is consonant with RTI-76 reducing V_{max}. Cocaine and nomifensine could thus either increase release or competitively inhibit uptake. Whether cocaine or nomifensine changed the inflection point of the clearance curve, indicative of altered K_m, was difficult to discern. The plot of drug-induced changes in $[DA]_{max}$ versus stimulation frequency was additionally revealing. Consistent with a change in K_m but not release, the effects of both cocaine and nomifensine were greatest at lower frequencies. The relationship between $[DA]_{max}$ and frequency was less steep for cocaine and nomifensine than for RTI-76. Taken together, the qualitative analyses suggest that the interaction between cocaine and nomifensine and DAT is similar and appears to be competitive. RTI-76, on the other hand, appears to be noncompetitive.

Quantitative kinetic analysis using curve fitting and the Wightman model demonstrated that cocaine and nomifensine significantly increased K_m for dopamine uptake but did not alter either V_{max} or $[DA]_p$ (Wu et al., 2001a). An identical conclusion was reached by single curve analysis (Wu et al., 2001b). RTI-76, in contrast, significantly decreased V_{max} (Wu et al., 2001a). The results of quantitative and qualitative approaches are therefore in excellent agreement: cocaine is a competitive inhibitor of DAT in vivo. Additionally, drug mechanism did not vary between striatal regions, but drug efficacy did depending upon uptake inhibitor. Both nomifensine and RTI-76 exhibited a greater effect on dopamine uptake in the nucleus accumbens compared to the caudate-putamen, whereas the effect of cocaine was similar.

Jones et al. (1995a) reached identical conclusions using a similar approach but in vitro. One advantage of slices is that high cocaine concentrations can be applied to alter the inflection point of the clearance curve. The electrically evoked signals in the presence of cocaine were well described by an increase in K_m, and the uptake inhibitor elicited similar effects in the caudate-putamen and nucleus accumbens. Nomifensine was also found to be a competitive inhibitor and less potent in the nucleus accumbens, again in excellent agreement with the in vivo study of Wu et al. (2001a).

Other voltammetric studies of cocaine–DAT interactions have yielded discrepant results. The most radically different is cocaine increasing the extracellular clearance rate of exogenous dopamine applied by pressure injection (Cass et al., 1993a,b). Other uptake inhibitors also appear to increase the clearance of exogenous (Zahniser et al., 1999) and electrically evoked (Stamford et al., 1986; Ng et al., 1992) dopamine. Studies with rotating disk electrodes, a voltammetric technique monitoring the clearance of exogenous dopamine from homogenized tissue or cells in suspension (Burnette et al., 1996; Earles and Schenk, 1998), concluded that cocaine is a noncompetitive inhibitor in the nucleus accumbens (Povlock and Schenk, 1997), an uncompetitive inhibitor in the caudate-putamen (McElvain and Schenk, 1992), and more potent in the nucleus accumbens than the caudate-putamen (Povlock and Schenk, 1997). Rotating disk electrode experiments with human embryonic kidney cells expressing human DAT found cocaine to inhibit dopamine uptake competitively (Earles and Schenk, 1999).

Identifying potential reasons for discrepancies between voltammetry studies, albeit difficult, is critical to further our understanding of cocaine–DAT interactions. One important consideration is kinetic analysis. Because uptake velocity is dependent on substrate concentration (Fig. 2C), reliance on any parameter that also varies with concentration (e.g., clearance rate, clearance time, half-life, half-width and amplitude) is risky. Determination of the concentration-independent K_m and V_{max}, or a first-order rate constant, avoids this problem.

An additional concern is distortion of kinetics by sensor response time and diffusion. Dynamics monitored at carbon-fiber microelectrodes coated with Nafion must be corrected. This cation-exhange polymer exhibits a diffusion coefficient for dopamine that is three orders of magnitude smaller than in brain extracellular space (Kawagoe et al., 1992). Although a bare carbon-fiber microelectrode instantaneously oxidizes dopamine when used with amperometry (i.e., constant-potential voltammetry), when used with FSCV, time-dependent changes in dopamine are distorted by surface adsorption and desorption (Venton et al., 2002; Bath et al., 2000). Diffusion, which has complex effects on kinetics, originates from two sources in the brain. The first is within a damaged region caused by sensor implantation. The size of this region is smaller than that caused by the much larger dialysis probe (Clapp-Lilly et al., 1999; Allen et al., 2001). The other source of diffusion is within intact tissue, as dopamine is clearly established as an extrasynaptic neurotransmitter (Vizi, 2000; Zoli et al., 1998).

Experimental design can lessen the detrimental effects of diffusional distortion but not completely eliminate them. Extracellular dopamine diffusion is minimized by electrical stimulation in vivo, because release is elicited throughout the dopaminergic projection field. Rapid mixing during rotating disk electrode measurements similarly limits diffusion. Mathematical models incorporating diffusion and uptake should improve the accuracy of kinetic analysis but have only been described for amperometry (Schonfuss et al., 2001; Schmitz et al., 2001; Venton et al., in press) not FSCV or rotating disk electrodes. On the other hand, administering dopamine as a point source either by pressure injection or iontophoresis (Gerhardt and Palmer, 1987) generates concentration gradients driving diffusion. DAT function can still be kinetically analyzed robustly under these conditions, but not without incorporating a diffusion term into the

model (Cragg et al., 2001; Nicholson, 1995; Cass et al., 1993a,b).

The electrical stimulation design for evaluating cocaine–DAT interactions is not without criticism. By evaluating uptake in the absence of release, experiments with exogenously applied dopamine are advantageous by not requiring mathematical models to resolve these two mechanisms. Electrical stimulation may also alter dopamine uptake. The activity of DAT is voltage dependent (Sonders et al., 1997; Zahniser et al., 1998), but uptake kinetics are slow compared to the fast voltage changes associated with an action potential (Prasad and Amara, 2001). There is the possibility that electrical stimulation changes the distribution of ions across dopaminergic neuronal membranes, driving dopamine uptake and determining the resting membrane potential. During experiments with electrical stimulation, sufficient time is allowed in between pulse trains permitting conditions for dopamine release to return to baseline. Uptake may recover similarly, because, like release, rates do not vary with pulse train order. Uptake also appears to be consistent within a pulse train, because one set of parameters describes the entire evoked signal.

Whether urethane, used to anesthetize animals in the Wu et al. (2001a,b) studies, affects cocaine-altered DAT interactions is not known. Urethane reduces electrically evoked dopamine release (Garris et al., 1997) and alters the time course of nomifensine effects on $[DA]_{max}$ (Garris et al., 2003). Michaelis–Menten parameters do not change with urethane (Garris et al., 2003), but chloral hydrate (Sabeti et al., 2000) and equithesin (Kiyatkin et al., 2000) have been shown to decrease dopamine uptake rates.

There is evidence that DAT blockers may enhance dopamine release (Cocchi et al., 1979; Raiteri et al., 1975). Although amphetamine is an established releaser by promoting reverse transport (Sulzer et al., 1995; Jones et al., 1998), the case for other inhibitors is less clear. Early studies measuring dopamine efflux into incubation media are criticized on the grounds that release and uptake mechanisms were not resolved (Garris and Ben Jonathan, 1991). A similar argument is made against the use of electrically evoked $[DA]_{max}$ as a measure of release (Stamford et al., 1989). However, voltammetric studies kinetically analyzing for a release parameter have found that cocaine exhibits dopamine releasing properties in vitro (Jones et al., 1995a; Lee et al., 2001). The mechanism mediating increased release is not established, thus considering this phenomenon, a cocaine–DAT interaction is premature. Cocaine did not increase $[DA]_p$ in the Wu et al. (2001a,b) studies, but drug effects were measured beginning 20-min post-administration, perhaps after releasing effects have subsided. The releasing effect of cocaine in vitro is observed initially following administration or as a rebound effect after drug withdrawal (Jones et al., 1995a; Lee et al., 2001).

4. Factors determining the enhancement of brain dopamine levels by cocaine

Cocaine preferentially increases extracellular dopamine levels in the nucleus accumbens compared to the caudate-putamen (Carboni et al., 1989; Kuczenski and Segal, 1992). This finding may be related to the key role played by mesoaccumbal dopaminergic neurons in mediating the behavioral effects of the drug (Wise, 1996; Kalivas and Nakamura, 1999; McBride et al., 1999). Interestingly, most studies show a similar efficacy for cocaine binding to DAT and inhibiting dopamine uptake in the two striatal regions (Boja and Kuhar, 1989; Cass et al., 1992; Izenwasser et al., 1990; Jones et al., 1995a; Wu et al., 2001a). As described in the previous section above, our analysis of electrically evoked dopamine dynamics also indicates that cocaine competitively inhibits dopamine uptake in both the nucleus accumbens and caudate-putamen. Taken together, these results suggest that the preferential increase in accumbal dopamine following cocaine administration is not due to unique cocaine–DAT interactions.

One emerging idea is that the nature of extracellular dopamine regulation determines the effect of cocaine on dopamine levels. Lower DAT activity, for example, is postulated to mediate the preferential effects of cocaine in the nucleus accumbens compared to the caudate-putamen (Cass et al., 1992) and the medial versus the lateral striatum (Cline et al., 1995). Other factors may be involved, because forebrain DAT activity is lowest in the cortex and amygdala (Jones et al., 1995b; Garris and Wightman, 1994) where cocaine-induced increases in extracellular dopamine are small (Moghaddam and Bunney, 1989; Hurd et al., 1997; Garris and Wightman, 1995). We have recently proposed that, in addition to DAT activity, rates for dopamine release must also be considered (Wu et al., 2001a).

The same kinetic analysis used to assess cocaine–DAT interactions yields pre-drug rates for dopamine release and uptake. These rates can be directly compared to the increases in extracellular dopamine levels produced by cocaine at the same recording location. When data from the caudate-putamen and nucleus accumbens are pooled, both dopamine release and uptake inversely correlate with cocaine effect (Wu et al., 2001a). This result demonstrates that lowest release and uptake rates are associated with greatest increases in extracellular dopamine following cocaine administration. Because, on average, the nucleus accumbens exhibits lower rates for dopamine release and uptake than the caudate-putamen, this result additionally provides a mechanism for the preferential effects of cocaine. Another important point to consider is that release and uptake rates are heterogeneously distributed in the striatum (Stamford et al., 1986; May and Wightman, 1989; Garris et al., 1994). Consequently, the combination of low dopamine uptake and release, and the enhanced effect of cocaine, also occur in sub-regions of the caudate-putamen (Wu et al., 2001a). Cocaine thus has

diverse effects on extracellular dopamine levels in the striatum.

Diffusion, another factor regulating extracellular dopamine, may also determine the effect of cocaine in sub-regions of the striatum. Michael and co-workers have demonstrated that the effects of nomifensine on electrically evoked dopamine levels are inversely related to the pre-drug concentration of elicited dopamine (Lu et al., 1998). In this voltammetric study, lower dopamine levels were attributed to microsensor placements at some distance from dopaminergic terminals, decreasing the released concentration by diffusion. There is no reason to suggest that the effects of cocaine and nomifensine would differ qualitatively. This same group also developed a mathematical model showing that the relative effect of an uptake inhibitor on extracellular dopamine levels increases exponentially with distance from a release site. Like release and uptake, moreover, the heterogeneous dopaminergic innervation also causes dopamine diffusion to vary in striatal sub-regions, suggesting that the diffusion enhancement of cocaine effects would be similarly diverse.

Voltammetric microsensors have not characterized dopamine release, uptake and diffusion across large areas of the striatum. Most voltammetric measurements, in fact, have been collected in microscopic regions of dense innervation or "hot spots". However, Venton et al. (in press) have recently simulated the relationship between these mechanisms and extracellular dopamine levels using parameters obtained from voltammetry for a one-dimensional strip of the striatum with a length of 200 μm. These calculations show that tonic firing of dopaminergic neurons produces spatially uniform dopamine concentrations. Steady-state dopamine levels result, because diffusion rapidly relaxes concentration gradients soon after release. In contrast, a short, synchronized burst of action potentials mimicking phasic signaling produces large differences in dopamine concentration with highest levels in regions of densest innervation.

The model of Venton et al. (in press) was also used to predict the effects of cocaine on these temporal and spatial dopamine dynamics in the striatum. Cocaine uniformly elicited increased extracellular dopamine levels in innervated and un-innervated regions alike during tonic signaling but enhanced concentration gradients for dopamine during phasic signaling. Unexpectedly, as dopamine was cleared by DAT in the innervated regions after a burst of release, dopamine pooled in the un-innervated regions in the presence of cocaine. These simulated results further underscore the diverse cocaine effects in the striatum.

5. Conclusions and future studies

Although conferring the advantage of whole animal measurements, evaluation of cocaine–DAT interactions by voltammetry is quite difficult. To overcome these problems, we coupled voltammetry to electrical stimulation and used a combination of quantitative and qualitative approaches for analysis. Taken together, the results indicate that cocaine competitively inhibits dopamine uptake in the nucleus accumbens and caudate-putamen in vivo. Voltammetry also suggests that the nature of extracellular dopamine regulation in a microscopic region determines the magnitude of cocaine effects on dopamine levels. Dopamine release, uptake and diffusion play a critical role in this regard. Because extracellular dopamine levels are heterogeneously regulated, cocaine is expected to elicit diverse effects throughout the striatum. What is not known is the neurobiological significance of these diverse effects on target cell activity. Future studies could address this important issue by combining voltammetry with electrophysiology.

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