

## Dynamic Observation of Dopamine Autoreceptor Effects in Rat Striatal Slices

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**Abstract:** Fast-scan cyclic voltammetry has been used to measure dopamine (DA) synaptic overflow in slices of rat caudate nucleus induced by electrical stimulation with one-, two-, and 50-pulse, 10-Hz trains. Synaptic overflow in this preparation is shown to be the result of the competing effects of release and cellular uptake. Release caused by all pulses was attenuated by the D<sub>2</sub> agonist quinpirole (1  $\mu$ M). The rapid time response of the measurements (100 ms) allows the autoinhibition induced by endogenous, released DA to be resolved in real time. The concentration of DA released during the second pulse of a train was 58% of that released by the first pulse, an effect that is partially blocked by the addition of 2  $\mu$ M sulpiride, a D<sub>2</sub> antagonist, to the perfusion buffer. DA release during the first stimulus pulse is unaffected by 2  $\mu$ M sulpiride, suggesting that autorecep-

tors are not normally occupied in this preparation. Release caused by the third pulse was 14% of the first pulse and also could be partially enhanced by 2  $\mu$ M sulpiride. The duration of the inhibition of release induced by endogenous DA was estimated by varying the interval between one-pulse stimulations until the overflow of DA induced by the second pulse was equal to that on the first; a half-time of  $\sim 17$  s was found. The addition of picrotoxin (100  $\mu$ M) and glutamate (10  $\mu$ M) to the perfusion buffer did not affect stimulated release of DA, although the addition of atropine (100  $\mu$ M) attenuated overflow for all the trains tested. **Key Words:** Dopamine—Autoreceptors—Striatum—Voltammetry—Quinpirole—Sulpiride. Kennedy R. T. et al. Dynamic observation of dopamine autoreceptor effects in rat striatal slices. *J. Neurochem.* 59, 449–455 (1992).

It is well established that neurotransmitter release from dopamine (DA) neurons can be modulated by several factors. For example, DA neurons in the substantia nigra, which project to the caudate nucleus, are known to contain autoreceptors, receptors that selectively recognize the substance released by these neurons (Starke et al., 1989). Activation of these receptors has many effects, including inhibition of DA synthesis, hyperpolarization of DA cell bodies (Roth, 1984), and inhibition of DA release (Starke et al., 1978; Cebeddu and Hoffmann, 1983; Dwoskin and Zahniser, 1986). Striatal slices have been shown to be particularly useful in investigating the effects of autoreceptors on release, because the terminal region can be studied in the absence of the cell body. Typically, the effects of autoreceptors on release have been investigated by measurement of the overflow of previously accumulated, radiolabeled DA into a perfusion buffer, and measurements usually have a temporal

resolution on the minute time scale. In some cases, endogenous DA overflow has been measured; however, it is necessary to use uptake inhibitors to obtain well-defined results (Cass and Zahniser, 1990). Taken together, these studies have shown that the DA autoreceptors exhibit a pharmacological profile indicative of the D<sub>2</sub> receptor.

Improvements in the measurement of DA overflow have allowed characterization of the temporal characteristics of autoreceptor effects. In the presence of an uptake inhibitor, the inhibition of tritiated DA overflow by DA autoreceptors has been characterized from perfused slices with the use of very brief electrical pulse trains (Mayer et al., 1988; Singer, 1988; Starke et al., 1989). These investigations showed that the DA overflow during the first pulse is unaffected by autoreceptors, whereas overflow caused by a train of pulses is inhibited relative to the first pulse because of interaction of the released ligand with its autorecep-

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**Abbreviations used:** DA, dopamine; [DA]<sub>s</sub>, the concentration of dopamine released per stimulus pulse; GABA,  $\gamma$ -aminobutyric acid; TTX, tetrodotoxin.

tor. Particularly noteworthy is the recent use of fast-scan cyclic voltammetry with carbon-fiber microelectrodes to observe the local overflow of endogenous DA inside a rat striatal slice after one electrical stimulation pulse (Bull et al., 1990; Palij et al., 1990). The amount of DA synaptic overflow in this preparation is sensitive to the stimulus intensity and to uptake inhibition. Furthermore, the overflow is inhibited by a D<sub>2</sub> agonist, an effect reversed by D<sub>2</sub> antagonists and not affected by D<sub>1</sub>-selective drugs.

The use of carbon-fiber microelectrodes implanted in neuronal tissue provides a convenient way to investigate the short-term dynamics of the overflow of DA with a spatial resolution of a few tens of microns (Stamford et al., 1986; Kuhr and Wightman, 1986; May and Wightman, 1989). When carbon-fiber microelectrodes are used with fast-scan voltammetry, a time resolution of 100 ms can be achieved, and the cyclic voltammogram gives evidence of the species detected (Millar et al., 1985). This technique gives the fastest measurements to date of DA levels in the caudate and provides a profile of release to go along with the well-described electrophysiological activity of DA cell bodies in the substantia nigra (Grace and Bunney, 1984). In previous work we confirmed the voltammetric measurements of DA overflow in slices and simultaneously, with the same electrode, examined oxygen consumption during electrical stimulation (Kennedy et al., 1992).

In the present study, we examine the temporal course of synaptic overflow during stimulations of different time courses and intensity. As will be shown, overflow measured during single-pulse stimulations with voltammetric electrodes in slices has quite different characteristics than overflow measured during 10-Hz pulse trains. During low-frequency trains it is possible to observe the rapid autoinhibition of subsequent DA synaptic overflow caused by the endogenous ligand released early in the stimulus train. The data presented herein represent the first time-resolved observation of autoinhibition of DA release.

## EXPERIMENTAL PROCEDURES

### Slices

Slices (350  $\mu$ m thick) were prepared from the caudate nucleus of male Sprague-Dawley rats using a Lancer Vibratome as previously described (Alger et al., 1984; Kelly and Wightman, 1987). The slices were submerged in a Scottsh-type chamber (Alger et al., 1984) and perfused with a Krebs buffer, preheated to 32°C, at 1 ml/min. The Krebs buffer consisted of 126 mM NaCl, 2.5 mM KCl, 1.2 mM NaH<sub>2</sub>PO<sub>4</sub>, 2.4 mM CaCl<sub>2</sub>, 1.2 mM MgCl<sub>2</sub>, 25 mM NaHCO<sub>3</sub>, and 11 mM dextrose and was saturated with 95% O<sub>2</sub>/5% CO<sub>2</sub>. The flow system was equipped with a two-position, six-port valve to allow buffer containing drugs to be applied to the slice.

### Electrochemistry

Nafion-coated carbon-fiber microelectrodes were prepared as described before (Kristensen et al., 1986). Fast-scan

cyclic voltammetry (Baur et al., 1988) used a commercially available potentiostat (EI-400; Ensmann Instrumentation, Bloomington, IN, U.S.A.). A sodium-saturated calomel electrode was used as a reference electrode, and all voltages are reported versus it. The electrode potential was linearly scanned from -400 mV to 900 mV and back to -400 mV at 300 V/s every 100 ms. This voltage range encompasses the oxidation of DA (at 500–700 mV) and the reduction of the quinone form of DA (at 0 to -200 mV). The current in the voltage range of the oxidation of DA is proportional to the DA concentration at the electrode. To obtain DA concentration versus time plots, the current at the oxidation potential for DA, obtained for each potential scan, is integrated and converted to concentration based on postcalibration of the electrode. Thus, the time plots have a resolution of 100 ms in the time axis. The time response of the electrode is determined in a flow stream (Kristensen et al., 1986) and was always  $\leq 200$  ms to reach 50% of maximal response.

The fast scan rates used generate a large background current, which can obscure the voltammetric current due to DA (Baur et al., 1988). To obtain cyclic voltammograms (current vs. voltage curves), the background current obtained before stimulation was digitally subtracted from the cyclic voltammograms of interest. Typically several individual scans were averaged together to obtain the background current and the signal current.

### Stimulation

Electrical stimulation was accomplished with a twisted-wire, bipolar electrode with 100- $\mu$ m tips (Plastic Products, Roanoke, VA, U.S.A.) separated by  $\sim 500$   $\mu$ m. Biphasic stimulation pulses, 2 ms wide at each phase, were generated by a locally constructed stimulator and optically isolated by Neuro Log system stimulus isolators (Medical Systems, Great Neck, NY, U.S.A.). The stimulating electrodes were mounted on a micromanipulator and positioned at the surface of the slice near its center as described previously (Kelly and Wightman, 1987). The working electrode, mounted on a separate micropositioner, was lowered to 75  $\mu$ m below the surface of the slice at a position 100–200  $\mu$ m from the center of the stimulating electrode pair. Electrode placements were done with the aid of a stereomicroscope (Bausch and Lomb, Rochester, NY, U.S.A.).

### Protocol

Evoked DA overflow was first determined by obtaining responses to electrical stimulation with the slices perfused with physiological buffer. Then, physiological buffer containing drug was applied for 20 min, and the responses were recorded again. Paired *t* tests were used to determine the significance of measured drug effects. Mean values are reported with the SEM. Where given, *n* refers to the number of slices, typically from different animals.

### Kinetic analysis

Quantitative analysis of synaptic overflow used a previously described kinetic model (Wightman and Zimmerman, 1990). The kinetics of uptake were determined from the rate of decay of the concentration of DA after the stimulation is terminated. The rate of disappearance should follow Michaelis-Menten kinetics (Near et al., 1988):

$$-(d[DA]/dt)_{\text{uptake}} = V_{\text{max}}/(K_m/[DA] + 1) \quad (1)$$

where  $V_{\text{max}}$  is the maximal rate of DA uptake and  $K_m$  is the Michaelis-Menten constant for uptake. The value for  $K_m$

was taken as  $0.16 \mu\text{M}$ , a value determined in synaptosomes prepared from rat striatal tissue (Near et al., 1988). The value of  $V_{\text{max}}$  is dependent on the location of the electrode and was determined experimentally from the descending slope following stimulation that resulted in a concentration of  $\text{DA} \gg K_m$  (typically a 40-Hz, 3-s stimulation).

The rate of synaptic overflow has been characterized by  $[\text{DA}]_p f$ , where  $f$  is the stimulation frequency and  $[\text{DA}]_p$  is a term that describes the concentration of DA released per stimulus pulse. Thus, during stimulation, the rate of appearance of DA is given by

$$(d[\text{DA}]/dt)_{\text{overflow}} = ([\text{DA}]_p f) - (d[\text{DA}]/dt)_{\text{uptake}} \quad (2)$$

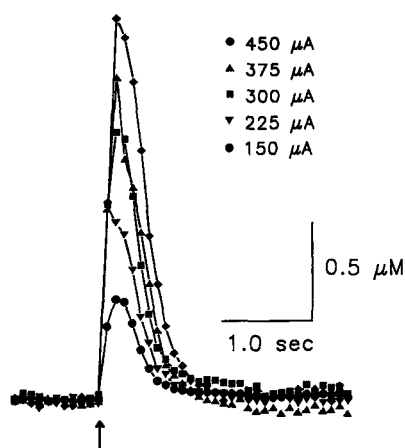
In these studies, the initial value for  $[\text{DA}]_p$  was determined directly from the amplitude of a one-pulse stimulation. The values for  $[\text{DA}]_p$ ,  $V_{\text{max}}$ , and  $K_m$  were then used with the integrated form of Eqs. 1 and 2 to simulate the response. In previous reports, the simulated response was modified to account for the response time of the electrode due to diffusion of DA through the Nafion film on the electrode (Wightman et al., 1988). For the data presented here, however, the Nafion films were sufficiently thin that the effect of diffusion was small enough to be negligible and was not included in the simulated response.

To account for changes in  $[\text{DA}]_p$  during the stimulus train, the following procedure was used. The second value of  $[\text{DA}]_p$  was determined by adjustment of the concentration to obtain a good simulation of a two-pulse stimulation while holding constant the value of  $[\text{DA}]_p$  for the first pulse,  $V_{\text{max}}$ , and  $K_m$ . Once the value for the second pulse was determined, the value for the third pulse, if present, was determined in a similar manner. For all of the 50-pulse, 10-Hz stimulations,  $[\text{DA}]_p$  did not require alteration beyond the fourth pulse to obtain a good fit to the data.

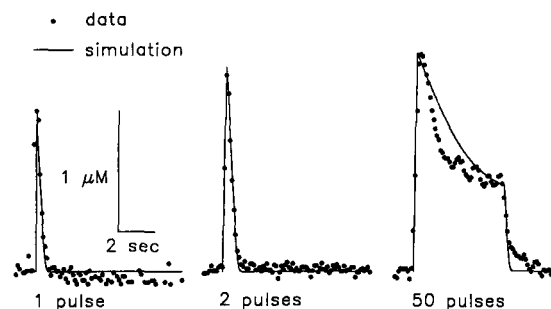
## RESULTS

### Effect of stimulation current magnitude

A rapid increase in DA concentration is observed following a one-pulse stimulation (Fig. 1). In all cases,



**FIG. 1.** DA overflow, measured by fast-scan cyclic voltammetry, as a function of stimulation current for one-pulse stimulations. Data points are the average current for DA oxidation measured in successive cyclic voltammograms. Stimulations were applied 5 min apart in a random order and are overlaid to facilitate comparisons. The voltammetric measurements were at 100-ms intervals. The arrow indicates the time of stimulus application.



**FIG. 2.** DA overflow (●) following stimulation with 300- $\mu\text{A}$  pulses with stimulus trains (10 Hz) of different durations. Simulations (solid lines) were based on variable  $[\text{DA}]_p$  values calculated as described in the text: first  $[\text{DA}]_p = 1.2 \mu\text{M}$ , second  $[\text{DA}]_p = 0.8 \mu\text{M}$ , and  $[\text{DA}]_p = 0.2 \mu\text{M}$  for pulses 3–50.  $V_{\text{max}}$  was  $3.5 \mu\text{M/s}$ , measured as described in the text. Data were obtained from the same slice with the same electrode positions. Recordings were made at 5-min intervals.

the maximal DA concentration is observed on the first or second measurement interval (50 or 150 ms after the pulse). Cyclic voltammetry was used to confirm the signal was due to DA. The DA concentration declines rapidly after the maxima and reaches a level below the detection limit (Wiedemann et al., 1991) within 1 s. The disappearance rate is nomifensine sensitive but unaffected by nialamide (Kennedy et al., 1992). The measured DA overflow was abolished in the presence of  $1 \mu\text{M}$  tetrodotoxin (TTX) or in the absence of  $\text{Ca}^{2+}$  (Kennedy et al., 1992). Both the TTX sensitivity and  $\text{Ca}^{2+}$  dependence of stimulated synaptic overflow are in agreement with previously obtained results using this technique (Bull et al., 1990; Palij et al., 1990). The maximal concentration observed increases with stimulus intensity. Whereas it was constant at a fixed position, the stimulation current required to obtain a particular amplitude showed variation within each slice. This is presumably due to imprecise electrode placement relative to stimulating electrodes and/or tissue heterogeneity. Normally, stimulation amplitudes between 200 and 300  $\mu\text{A}$  were used, which gave a readily detected signal.

### Effect of stimulus train length

Figure 2 demonstrates the effect of increasing the length of a stimulus train (10 Hz) on the observed time course of DA concentration. The average maximal DA concentration after one-, two-, and 50-pulse, 300- $\mu\text{A}$  stimulations was  $1.6 \pm 0.4$ ,  $2.1 \pm 0.5$ , and  $2.4 \pm 0.5 \mu\text{M}$  ( $n = 5$ ), respectively. Note that the amplitude following two-pulse stimulation is not twice that seen following a single pulse and that the concentration of DA actually decreases during the 50-Hz stimulation. The average height of the steady-state DA concentration after 50-pulse stimulations was  $0.6 \pm 0.1 \mu\text{M}$  ( $n = 5$ ).

To evaluate the amount released per stimulus pulse, the kinetic model was used. Typical results are

superimposed on the data in Fig. 2. One-pulse data were adequately modeled by assuming that the maximal observed concentration was equal to the release induced by the pulse and that its removal was by cellular uptake. The second value of  $[DA]_p$  had to be decreased to 56% of the first to model overflow adequately during two-pulse stimulations. To model the 50-pulse stimulations, the first three values for  $[DA]_p$  used for the simulation were successively decreased and then kept constant for the remainder of the stimulation interval (47 pulses), leading to a remarkably good fit. On average, the calculated second pulse was  $58 \pm 2\%$  ( $n = 6$ ) of the first pulse, and the calculated  $[DA]_p$  became constant by the third or fourth pulse at  $14 \pm 2\%$  ( $n = 6$ ) of the value of the first pulse. With this method, the average correlation coefficient ( $r$ ) for the agreement of the kinetic model to the data was 0.959.

### Effect of $D_2$ -selective drugs

Figure 3 shows typical stimulated DA synaptic overflow evoked by one- and two-pulse stimulations (10 Hz) and the effects of the  $D_2$  agonist quinpirole and the  $D_2$  antagonist sulpiride. The data demonstrate that sulpiride does not affect the amplitude or shape of the one-pulse stimulation. However, an increase in the concentration of DA is seen with two-pulse stimulations. In contrast, quinpirole decreased overflow on both one- and two-pulse stimulations.

The effects of quinpirole and sulpiride on the DA overflow after one- and two-pulse and the latter part of 50-pulse (10-Hz) stimulations were quantitatively evaluated and are summarized in Table 1. The pooled data bear out the fact that quinpirole inhibits DA release throughout the stimulation period. The inhibition of synaptic overflow was greatest for the single pulse. Sulpiride did not affect one-pulse overflow and

**TABLE 1.** Effect of  $D_2$  drugs on DA overflow following electrical stimulation

Drug (n)	% of control		
	$[DA]_{max}$ , 10-Hz, 1-pulse stimulation <sup>a</sup>	$[DA]_{max}$ , 10-Hz, 2-pulse stimulation <sup>b</sup>	$[DA]_{plateau}$ , 10-Hz, 50-pulse stimulation <sup>c</sup>
Sulpiride, 2 $\mu M$ (5)	105 $\pm$ 6	113 $\pm$ 4 <sup>d</sup>	318 $\pm$ 46 <sup>d</sup>
Quinpirole 1 $\mu M$ (5)	27 $\pm$ 4 <sup>d</sup>	45 $\pm$ 7 <sup>d</sup>	47 $\pm$ 6 <sup>e</sup>
10 $\mu M$ (4)	6 $\pm$ 4 <sup>d</sup>	13 $\pm$ 6 <sup>e</sup>	22 $\pm$ 9 <sup>d</sup>

Data are mean  $\pm$  SEM values of the indicated number of slices used (n).

<sup>a</sup> The maximal increase in DA concentration following one-pulse stimulation was measured in physiological buffer containing the drug of interest. The experimental data were collected 20 min after application of the agent. The measured value was divided by the control value (no drug present) and expressed as a percentage.

<sup>b</sup> Values shown were calculated as for the previous column except the DA concentration was the peak value after a two-pulse, 10-Hz stimulation.

<sup>c</sup> Values shown were calculated as for the previous column except the DA concentrations were taken from the steady-state region that developed during the latter part of 50-pulse, 10-Hz stimulations (see Fig. 2).

Significance of difference from the control: <sup>d</sup>  $p < 0.025$ , <sup>e</sup>  $p < 0.01$ .

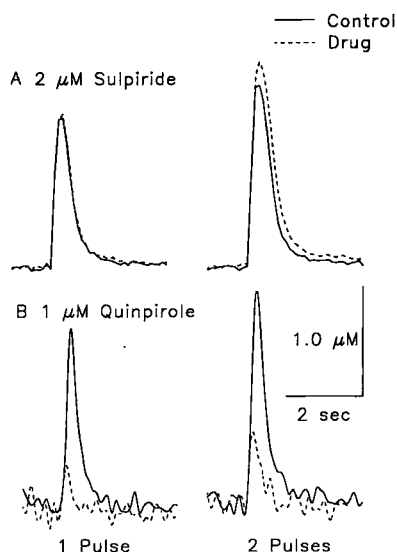
caused a modest, but significant, increase in the amplitude of synaptic overflow induced by two pulses. For both drugs, the effects are reversed by removal of the agent from the perfusion buffer.

### Effect of other pharmacological agents

Several other neurotransmitters are likely to be released during the electrical stimulation, and it is of interest to determine if they contribute to the shape of the DA overflow curve. The neurotransmitter systems tested were  $\gamma$ -aminobutyric acid (GABA), acetylcholine, and glutamate. Blockade of the  $Cl^-$  channel associated with the GABA receptor with picrotoxin did not affect the stimulated overflow of DA. Blockade of muscarinic receptors with atropine decreases DA overflow for one-pulse stimulations and for the entire period of a 50-pulse 10-Hz stimulation. The effect is reversed by removal of the drug. Glutamate did not affect DA overflow for any part of the stimulation. These results are summarized in Table 2.

### Recovery period

The time required between individual stimulation pulses to obtain a reproducible response was investigated. Two one-pulse stimulations were applied with sufficient time between them that the resulting overflow was baseline resolved. The time between stimulus applications was increased until the second response to stimulation equaled the first. The time between pairs of stimulations was at least 2 min. The results of a typical study are illustrated in Fig. 4, and the data from all the experiments are summarized in



**FIG. 3.** Effects of (A) 2  $\mu M$  sulpiride and (B) 1  $\mu M$  quinpirole on DA overflow elicited by one- and two-pulse (10-Hz) stimulations.

TABLE 2. Effect of other drugs on DA overflow

Drug (n)	% of control	
	[DA] <sub>max</sub> , 1-pulse stimulation	[DA] <sub>plateau</sub> , 50-pulse stimulation
Picrotoxin, 100 $\mu$ M (4)	110 $\pm$ 10	83 $\pm$ 12
Atropine, 100 $\mu$ M (6)	69 $\pm$ 8 <sup>a</sup>	54 $\pm$ 14 <sup>a</sup>
Glutamate, 10 $\mu$ M (4)	107 $\pm$ 5	101 $\pm$ 18

Data are mean  $\pm$  SEM values of the indicated number of slices used (n). The maximal increase in DA concentration following 10-Hz, one-pulse stimulation and the DA concentration from the steady-state region that developed during the latter part of 50-pulse, 10-Hz stimulations were measured in physiological buffer containing the drug of interest. The measured value was divided by the control value (no drug present) and expressed as a percentage.

Significance of difference from the control: <sup>a</sup>  $p < 0.05$ .

Fig. 4, inset. A full 60 s between stimulations is required to obtain recovery. The increase in the DA synaptic overflow from the second stimulus with increasing recovery time can be approximated by a double exponential in which the slower component has a half-life of  $\sim 17$  s. The addition of 2  $\mu$ M sulpiride to the buffer decreased the half-life of the slower phase to 5 s.

#### Synaptic overflow curves during 50-pulse stimulations

Although large changes in  $[DA]_p$  are seen during 50-pulse, 10-Hz stimulations, these curves are still sensitive to the effects of quinpirole and sulpiride (Table 1). Stimulated DA overflow during exposure to sulpiride also was simulated (Fig. 5). In the presence

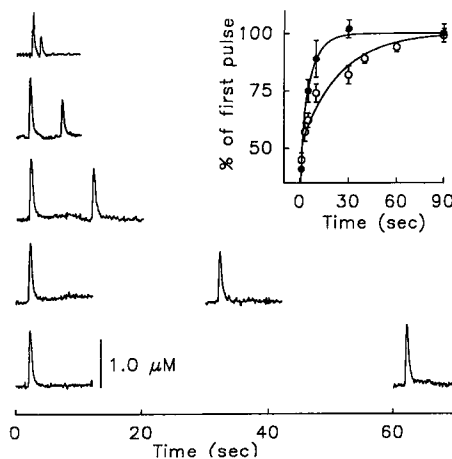


FIG. 4. Recovery of amplitude of DA overflow in response to one-pulse stimulations. A second pulse was given at 1, 5, 10, 30, or 60 s after the first pulse. Inset: Rate of recovery measured as the height of the second pulse relative to the first pulse for the control response ( $\circ$ ;  $n = 5$ ) and the response in the presence of 2  $\mu$ M sulpiride ( $\bullet$ ;  $n = 4$ ). Solid lines in the inset are double exponentials. For data with sulpiride present, the relative percentage was  $100 \times [ \frac{1}{2} \exp(-1.1t) + \frac{1}{2} \exp(-0.15t) ]$ . For control data the relative percentage was  $100 \times [ \frac{1}{2} \exp(-1.1t) + \frac{1}{2} \exp(-0.04t) ]$ .

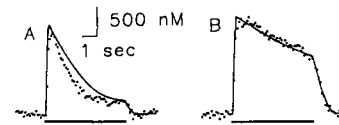


FIG. 5. Simulation (solid lines) of DA overflow compared with curves measured during 50-pulse, 10-Hz stimulations ( $\bullet$ ). Data are from the same slice and electrode positions.  $V_{max}$  was 2.0  $\mu$ M/s, measured as described in the text. A: Data obtained without added drug—first  $[DA]_p = 0.8$   $\mu$ M, second  $[DA]_p = 0.5$   $\mu$ M, and  $[DA]_p = 0.05$   $\mu$ M for pulses 3–50. B: DA overflow in the presence of 2  $\mu$ M sulpiride. For the simulation the first  $[DA]_p = 0.8$   $\mu$ M, second  $[DA]_p = 0.6$   $\mu$ M, and  $[DA]_p = 0.08$   $\mu$ M for pulses 3–50.

of 2  $\mu$ M sulpiride the second pulse was  $76 \pm 3\%$  ( $n = 4$ ) of the first pulse and became constant by the third to fifth pulse at  $22 \pm 7\%$  ( $n = 4$ ) of the first pulse (both values different from predrug values at the 95% confidence level).

#### DISCUSSION

In previous work with an *in vivo* preparation, we have shown that the synaptic overflow of DA into the extracellular fluid is a consequence of the imbalance between synaptic release and cellular uptake that occurs during stimulation (Wightman and Zimmerman, 1990). The results described here and in previous work (Bull et al., 1990; Palij et al., 1990; Kennedy et al., 1992) support a similar view for synaptic overflow in slices. The success in simulation of one-pulse data (Fig. 2), considering only uptake and release, shows that this simple view of overflow does a remarkable job in describing the observed results. However, the temporal profile of the DA concentration changes observed in striatal slices during 10-Hz stimulations shows that the factors that contribute to the observed overflow change considerably during the stimulation period. The changes that occur are quite rapid because the amplitude of DA overflow in a 10-Hz two-pulse stimulation is considerably less than twice that observed during a single pulse. Because the rate at which DA disappears from the extracellular space after the stimulation is similar irrespective of the stimulation length (see, e.g., Fig. 2), the data indicate that the concentration of DA released per stimulus pulse,  $[DA]_p$ , must decrease during the stimulation interval. The steady-state concentration that occurs during the latter part of a 50-pulse stimulation suggests that the amount of DA released per stimulus pulse eventually becomes constant but at a value less than the initial  $[DA]_p$ . Kinetic modeling provides support for this view. The modeling allows changes in  $[DA]_p$  to be discerned while simultaneously accounting for the effect of uptake; this is normally not possible from simple observations of the magnitude of synaptic overflow.

The effects of drugs known to have high potency at the DA  $D_2$  receptor, summarized in Table 1, demon-

strate that the changes in  $[DA]_p$  during the stimulus are mediated, at least partially, by  $D_2$  receptors. These results are consistent with prior reports (Bull et al., 1990; Palij et al., 1990) and, presumably, reflect the actions of autoreceptors located on DA neurons (Farnebo and Hamberger, 1973; Westfall et al., 1979). By such actions, DA released during the stimulation interacts with autoreceptors to inhibit DA released by subsequent pulses. Consistent with this view, blockade of the receptors with sulpiride has no effect on DA release induced by a single pulse. Because of this, the effects of sulpiride in two-pulse experiments appear modest because they only enhance release on the second pulse. This suggests that the receptors are not occupied by the endogenous ligand in the time between stimulus trains. Because the decrease in  $[DA]_p$  in the second pulse of the stimulation is attenuated by an antagonist, the data show that DA can partially inhibit its own release in subsequent pulses.

Consistent with the results with the antagonist, activation of  $D_2$  receptors by the agonist quinpirole significantly depressed the DA overflow as a result of single, double, and even prolonged pulse trains (see Fig. 3 and Table 1). The effect was most profound for the single stimulus pulse, where a 73% inhibition was observed with 1  $\mu M$  quinpirole. Thus, quinpirole exerts its greatest effect on  $[DA]_p$  when receptors are unoccupied (single-pulse stimulation) than on release that is autoinhibited (two-pulse and the latter part of 50-pulse stimulation).

The time resolution obtained with fast-scan cyclic voltammetry allows estimation of the time required for autoinhibition of DA release. The results with two-pulse stimulations show that  $[DA]_p$  is decreased on the second-stimulus pulse, a process that is sulpiride sensitive. Therefore, autoinhibition of DA release must begin within 100 ms. This conclusion agrees with previous measurements of  $[^3H]DA$  overflow from brain slices following high-frequency stimulus pulses, which suggested that DA autoreceptor activation began by  $\sim 40$  ms (Mayer et al., 1988). Full inhibition, resulting in a constant  $[DA]_p$ , occurs by the fourth stimulus pulse, that is, after 300–400 ms. The rapid rate at which maximal inhibition occurs shows that autoreceptors can operate effectively on a physiologically relevant time scale.

Single-pulse stimulations were used to examine the time for  $[DA]_p$  to return to its initial value. The recovery appears to be biphasic; it could be fitted to a biexponential (Fig. 4). A half-time of 17 s was found (Fig. 4) for the slower component. This time could be decreased by the addition of sulpiride to the medium. The sulpiride-sensitive component presumably reflects several factors, including dissociation of DA from the receptor and deactivation of second-messenger systems associated with the receptor. However, this result suggests that the actions caused by agonist activation of the  $D_2$  receptor are remarkably long-lived on a physiological time scale.

The failure of sulpiride to restore release completely during the second pulse to the value of the first suggests that other parameters also cause the apparent decrease in  $[DA]_p$ . Similar observations have been reported by Mayer et al. (1988) for release of DA as well as norepinephrine and acetylcholine. It may be that exogenous ligands may not be able to compete effectively with the endogenous, synaptic concentrations of released transmitter. Another possibility is that other endogenous neurotransmitters released by the stimulation may inhibit DA release. Treatment with a GABA-receptor antagonist did not alter any portion of DA-stimulated overflow. Glutamate, which can potentiate basal DA release in striatal slices (Krebs et al., 1991), also did not have an effect. Actions at *N*-methyl-D-aspartate receptors are blocked by  $Mg^{2+}$  at the concentrations used in this work. Atropine, a muscarinic antagonist, inhibited DA release uniformly. Because the first stimulus pulse is affected, it seems that stimulated release of acetylcholine does not cause the observed decrease. Effects of acetylcholine on DA overflow have been reported before and are consistent with our observations (De Belleruche and Bradford, 1978; Chesselet et al., 1982; Caudill et al., 1985).

The characteristics of  $[DA]_p$  in the slice preparation differ from those found in vivo. With medial fore-brain stimulation in the intact brain, striatal  $[DA]_p$  appears unchanged during stimulation trains and is considerably smaller (May et al., 1988). It is unclear whether these differences are a result of the mode of stimulation, the composition of the extracellular fluid [ $Ca^{2+}$  concentrations in the perfusion medium are twice the in vivo concentration (Moghaddam and Bunney, 1989)], or differences in temperature. Indeed, the conditions used in the present article appear to facilitate the observation of DA overflow on release.

The picture emerges of rapid, significant, and long-lasting inhibition of DA release by previously released DA acting at  $D_2$  receptors. This feature, combined with the rapid clearance of DA from the extracellular space because of cellular uptake, shows that synaptic DA will only exist at high concentrations for a transient interval. These conclusions are consistent with dialysis observations that released DA does not accumulate in the extracellular space (Moghaddam and Bunney, 1989). Thus, DA appears to act normally as a classical short-acting neurotransmitter in the caudate, in contrast to recent evidence from other brain regions that some neurotransmitters communicate by diffusing extrasynaptically (Fuxe and Agnati, 1991). Overflow is observed here, because all of the terminals are forced to fire in synchrony. Even under these extreme conditions, the extracellular concentration is tightly controlled. Note, however, that slight increases in  $[DA]_p$ , caused by sulpiride, lead to a dramatic increase in overflow during long stimulus trains (Fig. 5). Under these types of conditions, extrasynaptic communication will be much more likely.

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