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D_2-Like Dopamine Autoreceptor Activation Reduces Quantal Size in PC12 Cells

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D_2-like dopamine autoreceptors regulate dopamine release and are implicated in important actions of antipsychotic drugs and rewarding behaviors. To directly observe the effects of D_2 autoreceptors on exocytic neurotransmitter release, we measured quantal release of dopamine from pheochromocytoma PC12 cells that express D_2 and D_4 autoreceptors. High potassium-evoked secretion in PC12 cells produced a unimodal population of quantal sizes. We found that exposures to the D_2-like agonist quinpirole that inhibited tyrosine hydroxylase activity by ~50% also reduced quantal size by ~50%. The reduced quantal size was blocked by the D_2 antagonist sulpiride and reversed by L-DOPA. Quinpirole also decreased the frequency of stimulation-evoked quantal release. Together, these findings indicate effects on quantal neurotransmission by D_2-like dopamine autoreceptors previously distinguished as synthesis-modulating autoreceptors that regulate tyrosine hydroxylase activity versus impulse-regulating autoreceptors that modulate membrane potential. The results also provide an initial demonstration of a receptor-mediated mechanism that alters quantal size.

Key words: quantal size; PC12; dopamine; synaptic vesicle; amperometry; electrochemistry; D_2 receptors; quinpirole; sulpiride

D_2-like dopamine (DA) autoreceptors that inhibit stimulation-dependent DA release are present in mesencephalic DA dendrites (Saller and Salama, 1984; Nissbrandt et al., 1985; el Mestikawy and Hamon, 1986; Strait and Kuczenski, 1986) and presynaptic sites of mesolimbic terminals (Goldstein et al., 1990; Tepper and Groves, 1990; Westerink et al., 1990; Chen et al., 1991; Mercuri et al., 1992; Sesack et al., 1994). Because D_2-like autoreceptors are thought to play important roles in the action of antipsychotics (Hetey et al., 1991), psychostimulant drugs (Brodie and Dunwiddie, 1990; Bernardini et al., 1991; Seutin et al., 1991; Yamada et al., 1991; Giambalvo, 1992; Zhang et al., 1992; Pitts et al., 1993; Silvia et al., 1994; Xue et al., 1994), and sexual behavior (Hull et al., 1990), the mechanisms by which autoreceptors mediate synaptic DA release have received much attention. However, brain D_2-like receptors are located both presynaptically on DA afferents and postsynaptically on cortical and striatal neurons, making it difficult to isolate effects of autoreceptor activation on neurotransmitter release (Palij et al., 1990; Timmerman et al., 1990; Santiago and Westerink, 1991). Some reports suggest that D_2 autoreceptors on mesocortical DA afferents may primarily hyperpolarize membrane potential (impulse-regulating autoreceptors) (Chiodo et al., 1984; Bean and Roth, 1991; Fedele et al., 1993), whereas mesostriatal afferents and cell body autoreceptors may modulate both membrane potential (Bernardini et al., 1991; Cass and Zahniser, 1991; Kennedy et al., 1992; Tanaka et al., 1992; Cardozo, 1993) and the affinity of tyrosine hydroxylase (TH) for tyrosine (synthesis-modulating autoreceptors) (Bohmaker et al., 1989; Onali and Olianas, 1989; Salah et al., 1989; Johnson et al., 1992; Tissari and Lillgèls, 1993).

Recently, advances in electrochemical techniques have enabled the detection of vesicular release of monoamine transmitters in real time from chromaffin cells (Wightman et al., 1991; Chow et al., 1992), mast cells (Alvarez de Toledo et al., 1993), PC12 pheochromocytoma cells (Chen et al., 1994), invertebrate neurons (Bruns and Jahn, 1995; Chen et al., 1995), pancreatic cells (Zhou and Misler, 1996), mammalian sympathetic neurons (Zhou and Misler, 1995), and mammalian midbrain neurons (Pothos and Sulzer, 1998; Pothos et al., 1998). Zhou et al. (1994) have used these techniques to demonstrate a direct relationship between norepinephrine release and adrenergic autoreceptor activation in isolated chromaffin cells. Because PC12 cells express D_2-like receptors that can inhibit DA release after exposure to the agonist quinpirole (Courtney et al., 1991), we adapted this technique to examine the effect of D_2 autoreceptor activation on quantal DA release. We report that receptor activation reduces the amount of transmitter released per quantum, a previously unexplored potential mechanism of synaptic plasticity.

MATERIALS AND METHODS

PC12 culture. PC12 cells were obtained from Dr. Lloyd Greene (Columbia University) and maintained as described (Greene et al., 1991). For electrochemical experiments, 40,000 cells were plated per culture on glass coverslips (Carolina Biological Supply, Burlington, NC), coated with 40 μg/ml poly-D-lysine (Sigma, St. Louis, MO), and recoated with 10 μg/ml laminin (Collaborative Research, Bedford, MA). Cultures were maintained in media containing phenol red-free RPMI 1640 media supplemented with 10% heat-inactivated horse serum, 5% fetal bovine serum (JRH Biologicals, Lenexa, KS), and 50 U/ml penicillin and streptomycin (Life Technologies, Grand Island, NY) in 7.5% CO_2 atmosphere at 37°C. Experiments were performed on undifferentiated cells at least 5 d after plating, and compared cultures were derived from the same

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source culture. HPLC measurements of the cultures, which are grown in the absence of the DA β-hydroxylase cofactor ascorbic acid, indicate that <5% of catecholamines are norepinephrine and that the remainder is DA (Pothos et al., 1996).

RT-PCR methods. For total RNA extraction from PC12 cultures, a 60-mm-diameter tissue culture dish (Falcon) coated with 10 μg of Vitrogen-100 collagen/cm^2 (Collagen Biomedical, Palo Alto, CA) was plated with 2×10^6 PC12 cells. The cells were cultured for 1 week. The monolayer was rinsed with PBS, and 5×10^5 cells were harvested by trituration in 1 ml of the saline. One milliliter of Trizol reagent (Life Technologies) was added to the cell suspension. The tissue was homogenized by trituration through a 28.5 gauge needle, 200 μl of chloroform was added, and the RNA was precipitated from the aqueous phase after centrifugation (12,000 × *g* for 15 min) with isopropanol.

For total RNA extraction from brain tissue, 230 mg of tissue from rat ventral striatum was dissected from postnatal day 1 pups and washed in PBS. RNA extraction with Trizol reagent was performed as above, using 7 ml of Trizol and 1.4 ml of chloroform, and the tissue was homogenized by trituration with an 18 gauge needle.

mRNA from the total RNA sample was isolated using the Poly ATtract mRNA isolation system (Promega, Madison, WI) following the manufacturer's instructions. Reverse transcriptase and reagents for cDNA preparation from mRNA were obtained from the Superscript preamplification system kit (Life Technologies). Twenty microliters of the reaction mix were incubated at 42°C for 50 min, and the reaction was terminated by heating to 70°C for 15 min. The sample was then chilled on ice for 2 min, and the remaining RNA was degraded by incubation at 37°C for 20 min with RNase H.

For PCR amplification, we used custom primers (Life Technologies) with published sequences (Surmeier et al., 1996). For thermal cycling we used a Perkin-Elmer (Emeryville, CA) thermal cycler. Incubation times and temperatures were as follows: (1) 94°C for 4 min; (2) 45 cycles of 94°C (1 min), 56°C (1 min), and 72°C (1.5 min); and (3) 72°C for 10 min. Samples were then chilled at 4°C. Reagents were from Promega.

Tyrosine hydroxylase assay. To estimate the effects of drug exposure on TH activation in cultured cells, we adapted methods to measure DOPA formation (Hayashi et al., 1990). In brief, sister cultures were rinsed twice with 1 ml of the physiological medium used for the DA uptake assay (see below) and then incubated with DA agonists or antagonists for 40 min at 37°C in 100 μM L-tyrosine and 500 μM brocresine (Lederle Laboratories, Pearl River, NY), a selective DOPA decarboxylase inhibitor. After the incubation, medium was discarded, the cells were covered with 1 ml of 0.1N PCA, collected, and sonicated. An aliquot (50 μl) was used to determine protein concentration (Lowry assay), and the remainder was centrifuged at 15,000 × *g* at 4°C for 10 min. A 20 μl aliquot of the supernatant was injected onto a BAS Biophase ODS column (250 × 4.6 mm; 5 μm). Mobile phase consisted of (in mM): 50 potassium phosphate, 0.1 EDTA, 0.2 sodium octylsulfonate, and 10% methanol, pH 2.6. L-DOPA peaks were detected with an ESA Coulochem 5100A with a 5011 analytical cell.

DA uptake assay. We measured uptake using ^{3}H-labeled DA (Koide et al., 1986) in a physiological medium (in mM: 125 NaCl or choline chloride, 4.8 KCl, 1.2 potassium phosphate, 1.3 $CaCl_2$, 1.2 $MgSO_4$, 1 sodium ascorbate, 5.6 glucose, and 25 HEPES, pH 7.3). Sister cultures of PC12 cells were incubated for 40 min at 37°C in the appropriate physiological medium with or without receptor agonists and antagonists. The cultures were rinsed twice with physiological medium containing 125 mM NaCl for total uptake and 125 mM choline chloride for nonspecific uptake and then incubated in the same medium for 10 min at 37°C. This was replaced with the same medium containing a mixture of ^{3}H-labeled and unlabeled DA (0.3 nmol and 0.7 μCi/ml solution) for 5 min at 37°C. The reaction was stopped by placing the cultures on ice, followed by two 1 min rinses with ice-cold medium. Then, 2 ml of 1N NaOH were added to each dish, and the dishes were shaken overnight. An aliquot of the resulting solubilized mixture was mixed with 5 ml of scintillation cocktail, and radioactivity was counted using liquid scintillation. Specific uptake was determined by subtracting the nonspecific count and measuring total protein by the Lowry assay. DA uptake is expressed as picomoles per minute per milligram of protein.

Amperometric electrodes and apparatus. Carbon fiber electrodes were constructed by aspirating 5 μm carbon fibers (Amoco, Greenville, SC) into 1.2 × 0.68 mm glass capillary tubes (A-M Systems, Everett, WA) that were then pulled with a Flaming–Brown micropipette puller (Sutter Instruments, Novato, CA). The electrode tip was dipped into epoxy (Epo-Tek 301; Epoxy Technology, Billerica, MA) and cured at 100°C for 15 hr. The electrodes were back-filled with 3 M KCl. Electrode tips were polished at 40° on a beveler (World Precision Instruments, New Haven, CT). Electrode response was tested by cyclic voltammetry in a freshly prepared, nitrogen-bubbled 10 μM DA solution, and those with unstable *I–V* curves were rejected. The electrode was gently pushed against the cell body with a Huxley-style micropositioner (Newport Instruments, Irvine, CA).

A +700 mV voltage versus Ag–AgCl ground was applied to the carbon fiber electrode using an Axon 200A amplifier (Axon Instruments, Foster City, CA). The output was digitized at 50 kHz and low-pass-filtered at 10 kHz using an internal four-pole Bessel filter. The traces were digitally filtered at 2.5 kHz and analyzed using a locally written Superscope II program (GWI Instruments, Medford, MA). The average background current in the vicinity of the spikes was subtracted from the signal. Spikes were identified if their amplitude was 4.5 times greater than the rms background current, typically 0.3 pA. The number of molecules oxidized at the electrode face was determined by the relation $N = Q/nF$, where Q is the charge of the spike, n is the number of electrons transferred (shown to be two for catecholamines when used in a similar experimental configuration; Ciolkowski et al., 1994), N is the number of moles, and F is Faraday's constant (96,485 coulombs/equivalent).

For electrochemical recordings, physiological incubation medium contained (in mM):150 NaCl, 2 KCl, 1.2 $CaCl_2$, 1 $MgCl_2$, 1 NaH_2PO_4, 25 glucose, and 10 HEPES, pH 7.3. Drug exposures were for the times indicated at 37°C. The drug-containing medium or control medium was then removed and replaced with physiological incubation medium, and cells were recorded at room temperature (~25°C). The stimulation medium was composed of (in mM): 72 NaCl, 80 KCl, 6 $CaCl_2$, 21 glucose, and 10 HEPES, pH 7.3. The cells were stimulated by 6 sec superfusion of ~20 nl of stimulation medium at a distance of 18 μm from the recorded cell (Picospritzer; General Valve, Fairfield, NJ). Low pressure (<7–8 psi) was applied to avoid mechanical stimulation. Five second baseline durations were recorded before stimulation to identify spontaneously active cells. Two stimulations, spaced 20 sec apart, were administered. Interspike intervals and the *n* of events are only reported for the initial 20 sec period.

Statistics. Values are expressed as mean ± SEM. Kolmogorov–Smirnov statistics, exponential fits, regression analysis, and ANOVA were performed using GB-Stat (Dynamic Microsystems, Silver Springs, MD). Box diagrams were used to display the range of all mean values for the cell populations; median values were indicated as a point within the box, 25 and 75% values of the distribution were indicated by the box edges, and 10 and 90% values were indicated by the ends of the upper and lower lines. Cells that released less than five events within the 20 sec after stimulation were excluded from analysis. χ^2 values were determined by the formula [(observed − expected)^2/expected] with means as the expected values.

RESULTS

Identification of DA autoreceptor mRNA

There are three known D_2-like DA receptors, classified as D_2, D_3, and D_4 receptors (O'Dowd et al., 1994). To determine the expression of D_2 DA autoreceptors expressed in PC12 cells we used RT-PCR to identify cDNA transcripts, adapting methods used in the striatum (Surmeier et al., 1996). PC12 cells expressed message for D_2 and D_4 receptors at the predicted molecular weights (D_2 long and short forms, 404 and 317 bp; D_4, 223 bp) with no detectable D_3 message (Fig. 1). As a control, we also examined D_2-like mRNA in neonatal rat ventral striatum using the same primers. The striatum displayed D_3 message (461 bp) in addition to D_2 and D_4 message.

Effect of autoreceptor activation on TH activity

We recently found that the TH product L-DOPA increases quantal size in PC12 cells by increasing cytosolic DA synthesis (Pothos et al., 1996). Interestingly, TH activity can be modulated by a D_2 receptor-mediated pathway (Chiodo et al., 1984; Goldstein et al., 1990; Booth et al., 1994). To directly observe modulation of TH activity by D_2 activation, the TH product L-DOPA was measured in the presence of an aromatic acid decarboxylase inhibitor that

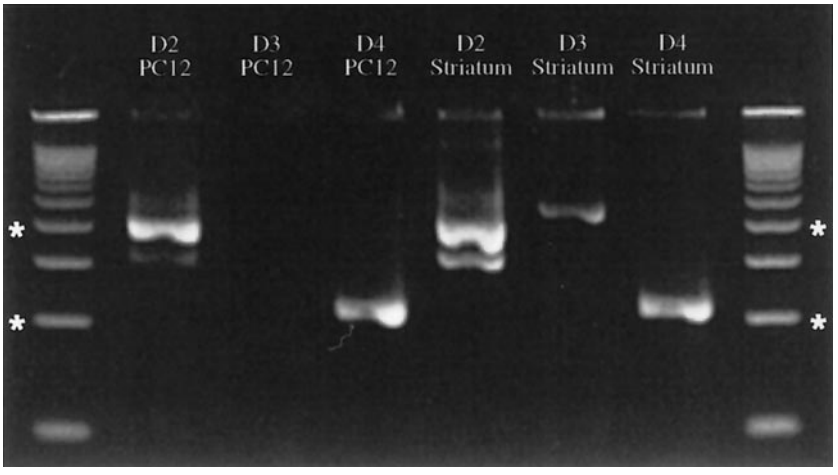

Figure 1. PCR analysis of D_2-like receptors in PC12 cells. RT-PCR analysis indicates that PC12 cells display message for D_2 (long and short forms) and D_4 DA receptors at the expected molecular weight (Materials and Methods). No D_3 message was observed. Ventral striatum (postnatal day 1) displays message for all three D_2-like receptors. The ladders on either side indicate 100 bp intervals for scaling. The 200 and 400 bp bands are indicated by *asterisks*.

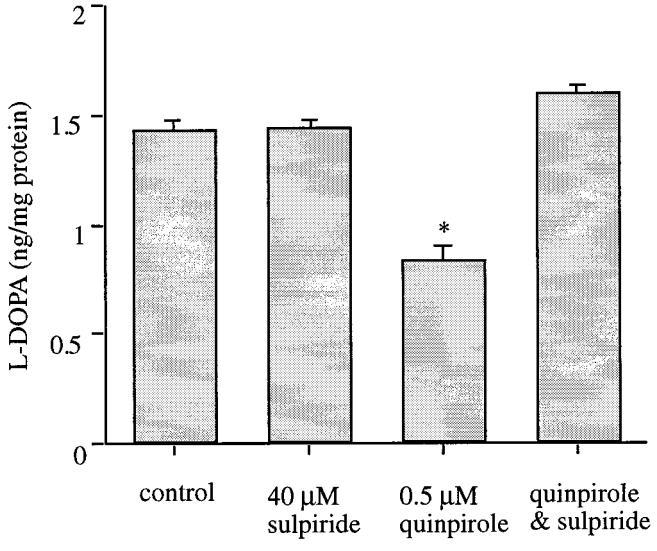

Figure 2. Effect of quinpirole on TH activity. PC12 cultures were exposed to tyrosine (100 μM for 40 min) in the presence of the DOPA decarboxylase inhibitor brocresine (500 μM). After incubation and extraction, L-DOPA was measured by HPLC. Quinpirole decreased L-DOPA synthesis to 59% of controls. The inhibition was blocked by coincubation with the D_2-like antagonist sulpiride (40 μM). $n = 5$ for each condition; error bars indicate SEM; $^*p < 0.0001$ by one-way ANOVA.

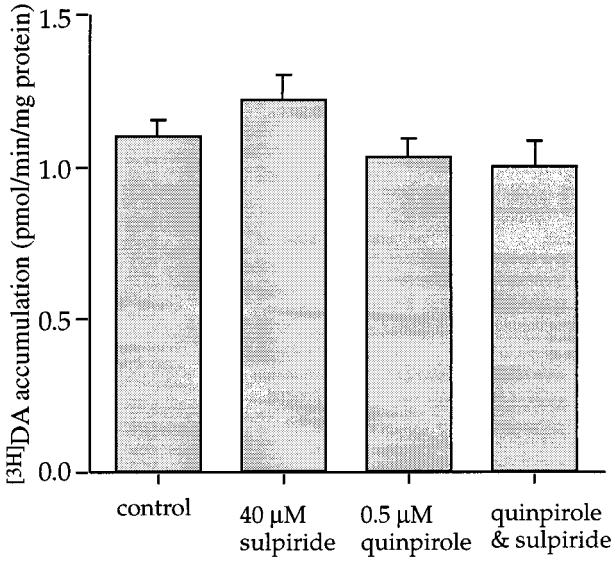

Figure 3. Effect of quinpirole on DA uptake. PC12 cultures were exposed to a combination of unlabeled and [^{3}H]DA (300 fmol) in physiological medium for 5 min. DA uptake was halted by replacing with ice-cold physiological medium, and the cells were solubilized with PCA. Background DA uptake was determined in saline in which choline replaced sodium. Exposure to either sulpiride or quinpirole did not alter specific DA uptake. $n = 5$ for each condition; error bars indicate SEM.

blocked L-DOPA conversion to DA (Arbogast et al., 1993). The D_2 agonist quinpirole (0.5 μM for 40 min) decreased TH activity to 59% of control levels (Fig. 2; $F_{(3,8)} = 35.5$; $p < 0.0001$ by one-way ANOVA). The effect of quinpirole was completely blocked by coincubation with the D_2 antagonist sulpiride. We chose this level of TH inhibition as appropriate for further study.

Effect of autoreceptor activation on DA uptake

DA uptake in synaptosomes (Krueger, 1990) and *Xenopus* oocytes transfected with DA transporter (Zhu et al., 1995; Sonders et al., 1997) is regulated by transmembrane potential. Therefore, another possible result of D_2 activation could be to modulate uptake by the plasma membrane catecholamine uptake transporter by altering ionic currents. We examined potential D_2 modulation of DA uptake by measuring uptake of low levels (300 fmol) of tritiated DA in the cultures; this low exposure would not saturate the transporter or significantly affect tonic extracellular DA in the cultures, which is ~10 nM (Pothos et al., 1996). We found that neither quinpirole nor sulpiride significantly altered accumulation of labeled DA (Fig. 3).

Quantal release from PC12 cells

To directly observe quantal release from undifferentiated PC12 cells, we used amperometric recording after low-pressure superfusion of high-K^+ medium. This resulted in current spikes (Fig. 4*A*) that were clearly resolved as individual events at an expanded time scale (Fig. 4*B*). Spikes were characterized by the number of molecules represented (molecules $\times 10^3$), amplitude (I_{max}), width at half-height ($t_{½}$), and the interval between successive events (int_{spike}) for those spikes within 20 sec after stimulation (Table 1). A low-amplitude "foot" was occasionally observed to precede the full current spike, particularly for higher-amplitude events (Fig. 4*C*), presumably because the events were not disguised in baseline noise. However, in all cases, the detectable feet contributed to <1% of release (Table 1). Such events have been observed in recordings of chromaffin, mast cell, and pancreatic

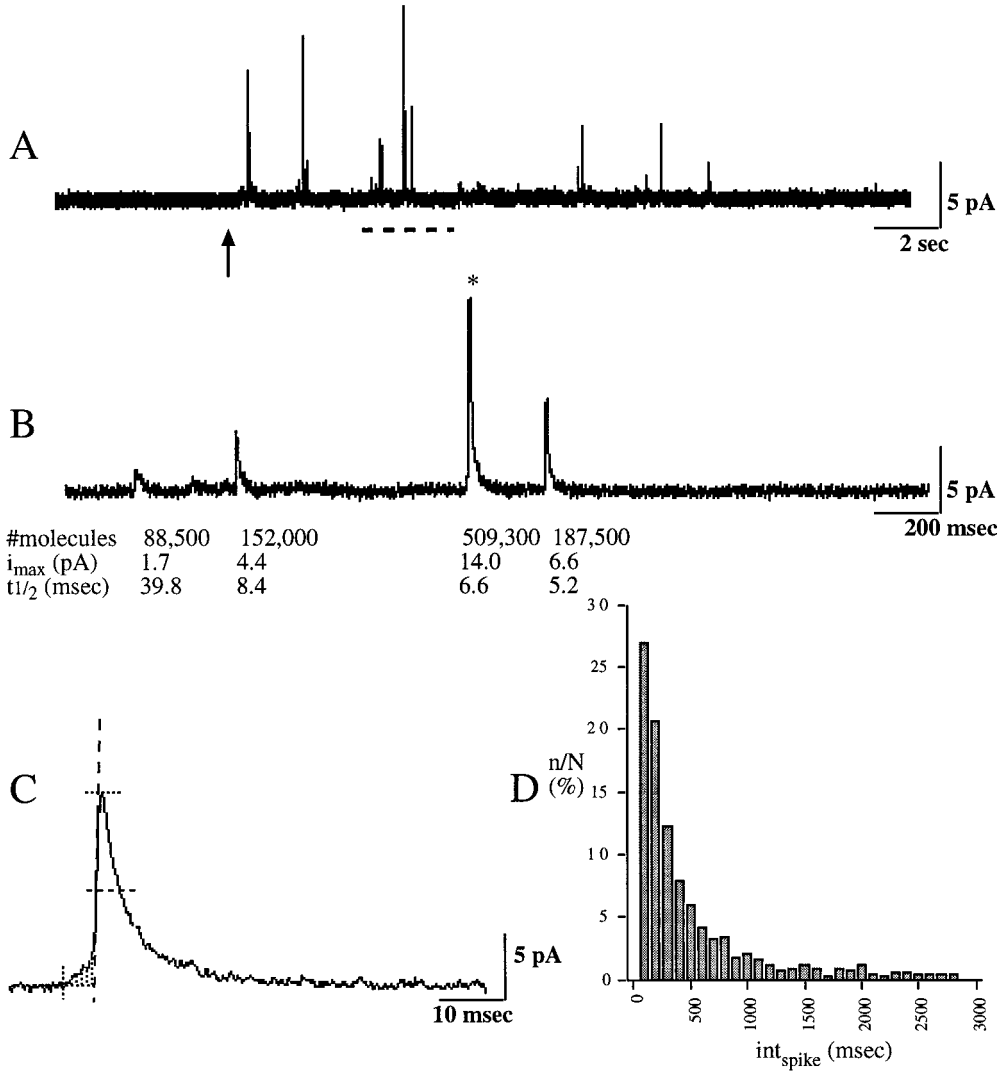

Figure 4. Depolarization by high potassium elicits quantal release in PC12 cells. *A*, Amperometric spikes after PC12 stimulation with 80 mM KCl for 6 sec (applied at *arrow*). This representative control trace represents 15 spikes ($I_{max} > 4.5$ rms noise), ranging from 1.25 to 14.0 pA with a mean amplitude of 4.8 ± 1.0 pA and quantal size of 201,000 ± 39,400 molecules. *B*, The portion above the *broken line* in *A* is displayed with an expanded time resolution. The quantal size (*#molecules*), peak amplitudes (I_{max}; in picoamperes), and width at half height ($t_{½}$; in milliseconds) are displayed under four corresponding events. *C*, For the sample event indicated with the *asterisk* in *B*, the maximal amplitude is indicated by the *top broken line*, and the half height is indicated by the *middle broken line*. The foot, filled by an array of points, is estimated by integrating the portion of the event preceding the slope (*broken line*) between the 60 and 90% rise-times (Chow and von Ruden, 1995). *D*, For control cells (events in Table 1), the interspike intervals (int_{spike}; in milliseconds) are displayed in 100 msec bins on the *x*-axis. The percentage of intervals within each bin (*n*/*N*) is labeled on the *y*-axis. The decreased representation at longer interval values is approximated by an exponential decay (see Materials and Methods).

β-cell exocytosis (Chow et al., 1992; Alvarez de Toledo et al., 1993; Zhou and Misler, 1996) and are suggested to represent transmitter release from an intermediate stage of fusion pore formation.

As expected, if release events were independent (Alvarez de Toledo and Fernandez, 1990), there was a good fit of int_{spike} values during the first 1500 msec after stimulation to an exponential decay [Fig. 4*D*; $y = (0.256)e^{(-0.0027/t)}$; $r^2 = 0.96$]. Given that the average spike width at half-height ($t_{½}$) in controls is 6.7 msec, the probability that two or more independent events would occur within this period can be estimated as:

$$1 - e^{-t/\tau} = 0.0179$$

where $t = 6.7$ msec; k = rate constant = 0.0027; and $\tau = 1/k = 370$ msec. Therefore, randomly overlapping spikes are rare.

Effect of D_2 autoreceptor activation on quantal release

In preliminary liquid chromatography experiments that confirmed the previous findings of Courtney et al. (1991), we found that incubation of PC12 cultures with 0.5 μM quinpirole for 25 min reduced DA release in high-potassium saline to 54 ± 13% of control levels, and that this effect was completely inhibited by 40 μM sulpiride (data not shown).

As an initial approach, we analyzed quantal release by examining the response of individual cells to successive stimulations (80 mM K^+, 6 sec) at 0, 5, 15, 25, and 35 min in the presence of normal recording medium or 0.5 μM quinpirole. Although >90% of PC12 cells displayed quantal release after the first stimulation, only 19% (4 of 21 cells recorded) of the control cells that responded to the first stimulation responded to a second stimulation 5 min later. This was further reduced at three subsequent 10 min intervals to 14% (3 of 21) of control levels at 15 min after the first release, to 10% (2 of 21) at 25 min, and to 5% (1 of 21) at 35 min; the decrease was significant at $p < 0.0001$; $\chi^2 = 44.97$. Moreover, the number of events per stimulation decreased in successive stimulations (mean, 40 events per cell at 5 min, 7 events at 15 min, 4 events at 25 min, and 3 events at 35 min; the decrease was significant by ANOVA with repeated measures ($F_{(4,54)} = 4.597$; $p < 0.01$). Similar results were found for quinpirole-treated cells (data not shown). We conclude that although the recording configuration is stable, refill of the releasable vesicle pool in PC12 cells is very slow. This contrasts with bovine adrenal chromaffin cells, which, as we and others have observed, continue to exhibit stable release after numerous stimulations (Finnegan et al., 1996). This appears to be because bovine chromaffin cells are densely

Table 1. Average characteristics of quantal events

	Quantal size	I_{max}	$t_{1/2}$	int_{spike}	% with foot	Foot size	*n*	*N*
Control	173,300 ± 8700	5.4 ± 0.2	6.7 ± 0.2	598 ± 43	3.1%	30,600 ± 5100	2316	48
0.5 μM quinpirole	88,600 ± 3200	4.7 ± 0.2	5.7 ± 0.2	827 ± 65	3.3%	17,600 ± 3700	718	24
40 μM sulpiride	183,400 ± 8400	6.8 ± 0.4	6.8 ± 0.2	566 ± 41	6.8%	39,000 ± 7900	1270	30
Quinpirole/sulpiride	157,000 ± 9600	6.8 ± 0.7	6.4 ± 0.3	551 ± 79	8.2%	22,600 ± 3900	821	15
50 μM L-DOPA	428,000 ± 22,500	9.8 ± 0.5	7.2 ± 0.2	562 ± 56	7.1%	59,300 ± 16,000	1329	26
Quinpirole/L-DOPA	368,700 ± 17,900	6.7 ± 0.2	7 ± 0.3	557 ± 59	3.2%	126,000 ± 64,300	840	24

Quantal size refers to the number of molecules per event, I_{max} to the maximum amplitude (in picoamperes), $t_{1/2}$ is the duration at half maximum amplitude (in milliseconds), int_{spike} is the interval between events (in milliseconds), % with foot is the percentage of events that display a detectable foot (minimum 1000 molecules), and foot size is the number of molecules represented within the foot. The number of events per group (*n*) and number of cells recorded per group (*N*) are shown. Experimental groups were exposed to 0.5 μM quinpirole, 40 μM sulpiride, or 50 μM L-DOPA as indicated for 40 min before recording. Mean ± SEM are reported. For sulpiride treatments, events from cells that exhibited more than one spontaneous quantal event before stimulation were excluded from the analysis (434 events from 9 cells) so that the total data recorded in this category represented $n = 1704$ events from 39 cells. If the additional data from these spontaneously releasing cells is included in the analysis, the quantal size, I_{max}, $t_{1/2}$, % with foot, and foot size are nearly identical (data not shown).

filled with granules that provide rapid replacement of docked vesicles (Steyer et al., 1997), whereas PC12 cells have a smaller presence of mobile granules, requiring increased time for replenishment of the releasable pool (Burke et al., 1997).

An alternate successful approach was to compare bins of large groups of quanta from multiple cells after single applications of secretagogue, particularly because the quantal size distribution was found to fit a lognormal distribution. To determine the population distribution of quantal parameters in PC12 cells, we examined 2316 quantal events elicited from 48 control cells (Table 1). As reported previously, untransformed PC12 quanta and other amperometrically detected quanta do not show a normal size distribution but are skewed to the right (Fig. 5*H*, *left*). Earlier studies have shown that either a cubed root or log transformation of the quantal amplitudes of postsynaptically recorded quanta (Van der Kloot, 1991) or amperometrically recorded quantal sizes (Finnegan et al., 1996) results in a normal distribution. The suggested rationales are that (1) vesicle diameters are distributed normally; therefore, if transmitter filling is proportional to volume, the population distribution should produce a cubed root normal distribution (Bekkers et al., 1990); and (2) if multiplicative deviations from the mean occur, this would produce a lognormal distribution (Van der Kloot, 1991); an example would be if integral differences in the number of uptake transporters per vesicle resulted in multiplicative transmitter accumulation rates. In the present case, we found that the lognormal transformation resulted in a closer fit of the individual values to a normal probability function than the cubed root transformation (Table 2). Therefore, the quantal size populations are lognormal and unimodal. Although a small number of values at the extreme edges are not well described by this distribution, they are typically <1% of the events. In the case of larger events that deviate from the lognormal distribution, this may be attributable to overlapping multiple events. In a second statistical test of the normal distribution of quantal sizes, the Kolmogorov–Smirnov (K–S) test of normality showed a close fit of the lognormal distribution to a Gaussian distribution (K–S Z score = 2.0216; $p < 0.05$).

By comparing binned quantal sizes from multiple cells, we found that quinpirole reduced the average quantal size to 51% of control values (K–S $Z = 2.886$; $p < 0.0001$; Fig. 5*B*, Table 1), close to the inhibition of TH reported above. The distribution of the mean quantal sizes from each cell measured is indicated (Fig. 5*C*). Quinpirole was also observed to decrease the maximum amplitude, width at half-height, and number of molecules represented by the foot when detected (Table 1). We reproduced this experiment four additional times and observed quinpirole-induced reduction of quantal size to 49, 54, 62, and 74% of control levels. In an experiment in which cells were exposed to 0.5 μM quinpirole for 150 min rather than 40 min, quantal size was reduced to 31% of control levels (K–S $Z = 4.499$; $p < 0.0001$; $n =$ 144 events from 13 control cells; $n = 169$ events from 25 quinpirole-treated cells).

To examine whether the effect of D_2-like activation on quantal size could be blocked, we used the D_2-like antagonist sulpiride. Sulpiride (40 μM) alone had no effect on quantal size but blocked the effects of quinpirole (K–S $Z = 3.619$; $p < 0.0001$); the combination of quinpirole and sulpiride was not different from controls (Table 1). In preliminary experiments, we found that lower concentrations of sulpiride (2 and 10 μM) produced only partial blockade (data not shown) (the affinity of quinpirole is 3.9 nM for D_2 and ~12 nM for D_4; the affinity for sulpiride is 14.7 nM for D_2 and 52 nM for D_4; O'Dowd et al., 1994).

Quinpirole also had a significant (K–S $Z = 2.258$; $p < 0.0001$) effect on the frequency of release events as measured by the interspike intervals (Fig. 5*D*; Table 1). The reduction in frequency was also blocked by sulpiride (K–S $Z = 2.756$; $p < 0.0001$; Fig. 5*E*,*G*). Sulpiride and sulpiride–quinpirole treatments were not significantly different from controls, although sulpiride exposure induced spontaneous release in a fraction of PC12 cells, nearly the only instances of this observed. Of the cells recorded after sulpiride exposure, 23% (9 of 39) showed more than one release event in a 5 sec period before application of the secretagogue; these were excluded from the analysis of interspike intervals (Fig. 5, Table 1). The effect of sulpiride on release suggests that low tonic levels of DA in these cultures may serve to block spontaneous release by D_2-like receptor activation.

Effects of L-DOPA on quinpirole action

If quinpirole reduces quantal size by inhibiting TH, the effect should be reversed by the TH product L-DOPA. We exposed PC12 cultures to 50 μM L-DOPA for 30 min, which elevated the average quantal size by 250% (Fig. 6*G–J*; the total population was increased, K–S $Z = 14.628$; $p < 0.0001$) as observed in a previous study (Pothos et al., 1996). The L-DOPA and L-DOPA–quinpirole treatments did not produce different effects. The combination of L-DOPA and quinpirole increased the average quantal size over controls by 213% (K–S $Z = 12.631$; $p < 0.0001$) and the average quantal size over quinpirole-only treatments by 416% (K–S $Z =$ 5.508, $p < 0.0001$). This effect is consistent with inhibition of L-DOPA synthesis by D_2 activation and also demonstrates that

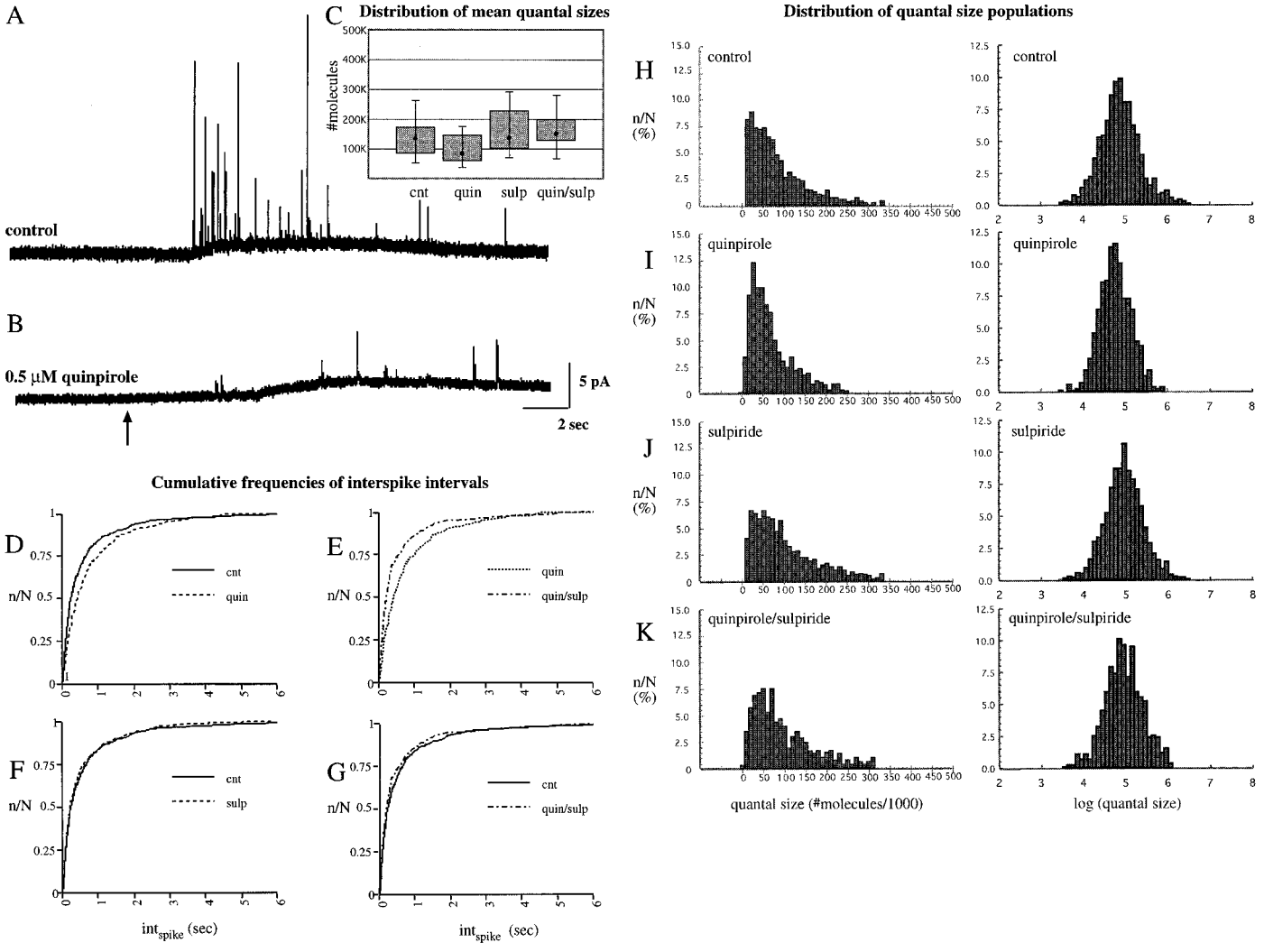

Figure 5. Effects of quinpirole on quantal release. Examples of traces recorded from (*A*) a control cell and (*B*) a cell exposed to 0.5 μM quinpirole for 40 min. Cells were stimulated by 6 sec applications of 80 mM KCl at the *arrow*. Quinpirole decreased the mean quantal size of individual release events (Table 1). *C*, Distribution of mean quantal size values for all cells that displayed more than five events within 20 sec of stimulation (controls, $n = 34$; quinpirole, $n = 18$; sulpiride, $n = 30$; quinpirole/sulpiride, $n = 13$). *D–G*, The cumulative frequencies of interspike intervals are compared between treatments. Quinpirole shifted interspike values to longer durations. The effect was blocked by sulpiride (*B*). *H–K*, For each group, the distribution of quantal sizes (*#molecules/1000*) is indicated as a histogram of the untransformed quantal sizes (*left column*) and the log values of those quantal sizes (*right column*). Quinpirole shifted the frequency distribution of quantal sizes to the left. The lower limit of each bin size is displayed on the *x*-axis. The distribution of log transformations in each case is closely approximated by a normal distribution ($r^2 \geq 0.987$; Table 2).

quinpirole does not reduce quantal size by blocking vesicular uptake of cytosolic transmitter. Surprisingly, L-DOPA blocked the decreased frequency of release elicited by quinpirole ($p < 0.0001$; K–S $Z = 3.3015$; L-DOPA–quinpirole was not different from controls; Fig. 6*D–F*).

Although it might be assumed that DA itself would have quinpirole-like effects, we found that 50 μM DA actually elevated quantal release (425,400 ± 18,300 molecules; $n = 1691$ events recorded from 49 cells), to levels similar to 50 μM L-DOPA. We presume that whereas DA itself is an effective agonist at D_2 receptors, it has multiple actions including activation of D_1-like receptors, which are also present in PC12 cells (Inoue et al., 1992). In addition, it is a substrate for the plasma membrane catecholamine uptake transporter of these cells (Ramachandran et al., 1993) and the vesicular monoamine transporter VMAT1 (Liu et al., 1994) and thus would be loaded into the vesicles. Therefore, DA activation of D_2-like receptors does not provide a straightforward approach to analyzing D_2-mediated alteration of quantal size, mostly because of the elevation of cytosolic stores of DA. We also found that including sulpiride (40 μM) with DA had no effect on the DA response (460,100 ± 20,400 molecules; $n = 1365$ events from 30 cells) and did not unmask D_2-like mediated effects on quantal size.

DISCUSSION

Modulation of synaptic neurotransmitter release can be attributable either to effects on the number of quanta released or the quantal size. The activation of a variety of neurotransmitter receptors is known to alter the frequency of quantal release events (Thompson et al., 1993). Although autoreceptors inhibit stimulation-dependent neurotransmitter release from a range of secretory cells, it is unclear whether such effects are attributable solely to a reduction in the number of quanta released, or if reduced quantal size might also play a role. Here we report clear

Table 2. r^2 values for best fit of normal probability distributions

	Lognormal	Cubed root	Untransformed
Controls	0.987	0.831	0.318
0.5 μM quinpirole	0.998	0.951	0.680
40 μM sulpiride	0.996	0.905	0.462
Quinpirole/sulpiride	0.997	0.949	0.688
50 μM L-DOPA	0.994	0.880	0.416
Quinpirole/L-DOPA	0.997	0.925	0.553

Linear best fit regressions were calculated for normal probability distributions of raw values and cubed root and log transformations of the quantal sizes. The population distributions are shown in Figures 5 and 6. In each case, the data points were better fit by a lognormal transformation than a cubed root transformation.

evidence indicating that autoreceptor activation can reduce quantal size.

We are aware of four mechanisms by which D_2-like autoreceptors might mediate apparent quantal size: (1) D_2 activation could decrease the occurrence of simultaneous release events that are misidentified as single quanta; (2) D_2 activation could induce morphological changes resulting in an increased distance of release from the electrode, causing the spikes to appear smaller because of diffusion; (3) D_2 activation could promote release of a subpopulation of vesicles with lower levels of transmitter; and (4) by inhibiting DA synthesis or vesicular accumulation, autoreceptors could reduce the total amount of neurotransmitter sequestered within vesicles and hence the number of molecules released during the expression of the fusion pore.

Our results suggest that overlapping events do not explain altered quantal size, because the exponential decay of the distribution of the interspike intervals suggests that <2% of spikes in the control preparations would overlap. The results are also inconsistent with release from more distal sites. In the protocol used in this study, the electrode was placed directly on the cell surface. If amperometric current spikes were not directly under the electrode, they would have shorter amplitudes and increased durations because of DA diffusion (Schroeder et al., 1992); however, quantal release from quinpirole-treated cells exhibited shorter amplitudes and decreased duration. Release of a smaller subpopulation of vesicles is inconsistent with the unimodal lognormal distribution of the populations. Although PC12 cells have both small (~50 nm diameter) and large (~150 nm diameter) vesicles, the small vesicles typically contain acetylcholine rather than DA (Schubert et al., 1980; Bauerfeind et al., 1993), which would not be detected. Our data support the hypothesis that quinpirole decreases quantal size by reducing the amount of DA packaged and released per vesicle. A difference in transmitter packaging is suggested by the leftward shift of the cubed roots of quantal sizes, which is consistent with a reduction of the amount of DA per vesicle volume. Moreover, this finding is congruent with decreased release from an entire culture, as previously reported (Courtney et al., 1991), confirming a genuine reduction of DA release.

Mechanism of altered quantal size

A potential mechanism by which D_2-like receptor activation might alter the amount of DA packaged per vesicle is by modulation of the activity of VMAT1. VMAT1 has been reported to be inhibited by cAMP-dependent phosphorylation (Nakanishi et al., 1995); however, the effect we observed is in the opposite direction of that which would be predicted after D_2 activation, which decreases intracellular cAMP (Goldstein et al., 1990).

An alternate possibility stems from the idea that, because vesicular transmitter accumulation is dependent on the transvesicular electrochemical gradient and substrate concentration gradient, D_2 receptor activation might reduce the available pool of cytosolic DA. We examined two mechanisms that could underlie altered cytosolic pools, decreased reuptake and decreased synthesis. We found that although D_2 activation has been found to modulate DA reuptake (McElvain and Schenk, 1992; Meiergerd et al., 1993; Cass and Gerhardt, 1994; Wieczorek and Kruk, 1994), under the conditions used, quinpirole did not inhibit the uptake of labeled extracellular DA by the PC12 catecholamine transporter.

The second mechanism addressed was suggested by a large body of evidence suggesting that D_2 receptor activation inhibits DA synthesis by blocking cAMP-dependent PKA-mediated phosphorylation of TH. PKA-mediated phosphorylation of the serine 40 residue activates TH, whereas reduced PKA activity after D_2 autoreceptor activation causes a reduction in ser40 phosphorylation (Harada et al., 1996). The reduced phosphorylation of ser40 in turn increases binding affinity of catecholamines to the iron moiety of TH resulting in a feedback inhibition of enzymatic activity (Griffith and Schulman, 1988; Haycock and Haycock, 1991; Daubner et al., 1992; Kumer and Vrana, 1996). Consistently, D_2 autoreceptor effects have long been known to inhibit DA synthesis (Chiodo et al., 1984; Goldstein et al., 1990; Booth et al., 1994). The quinpirole exposures we used inhibited TH activity to nearly the same level as the reduction in quantal size. Therefore, we suggest that D_2 activation decreases quantal size by inhibiting TH activity and reducing cytosolic DA available for vesicle accumulation.

We also noted a decreased quantal release frequency after D_2 activation that was blocked by sulpiride. Surprisingly, L-DOPA also blocked the quinpirole-mediated decrease in frequency. The mechanism by which L-DOPA produces this response is not clear. It is possible that, rather than a reduction in the rate of exocytosis, vesicle fusion after quinpirole proceeds at the normal rate but includes quanta with small amounts of transmitter that are below detection. These empty vesicles might then be filled if elevated cytosolic DA were available after L-DOPA. However, the ability of sulpiride to promote spontaneous release without a change in quantal size is consistent with a D_2 influence on vesicle exocytosis. Additional potential explanations for the L-DOPA reversal of the effects of quinpirole on frequency are that very high levels of L-DOPA act as an antagonist at D_2-like receptors or that upregulated DA after L-DOPA provides additional effects. An elegant approach to this in the future may be provided by simultaneous patch capacitance and amperometric recording of monoquantal release (Albillos et al., 1997).

Cellular and behavioral effects

Recent work indicates that the quantal size of catecholamine release can be regulated by various pharmacological interventions. (1) Transmitter accumulation is dependent on the magnitude of the vesicular electrochemical gradient. Amphetamine reduces the quantal size of DA release by collapsing the vesicular proton gradient (Sulzer et al., 1995). (2) Quantal size can be increased by elevating cytosolic neurotransmitter levels, and L-DOPA increases the quantal size of DA release (Pothos et al., 1996, 1998). (3) We now provide evidence that autoreceptor-mediated inhibition of neurotransmitter synthesis decreases the quantal size of DA release. More generally, although quantal size is often modeled as invariant (cf. Van der Kloot, 1991; Frerking

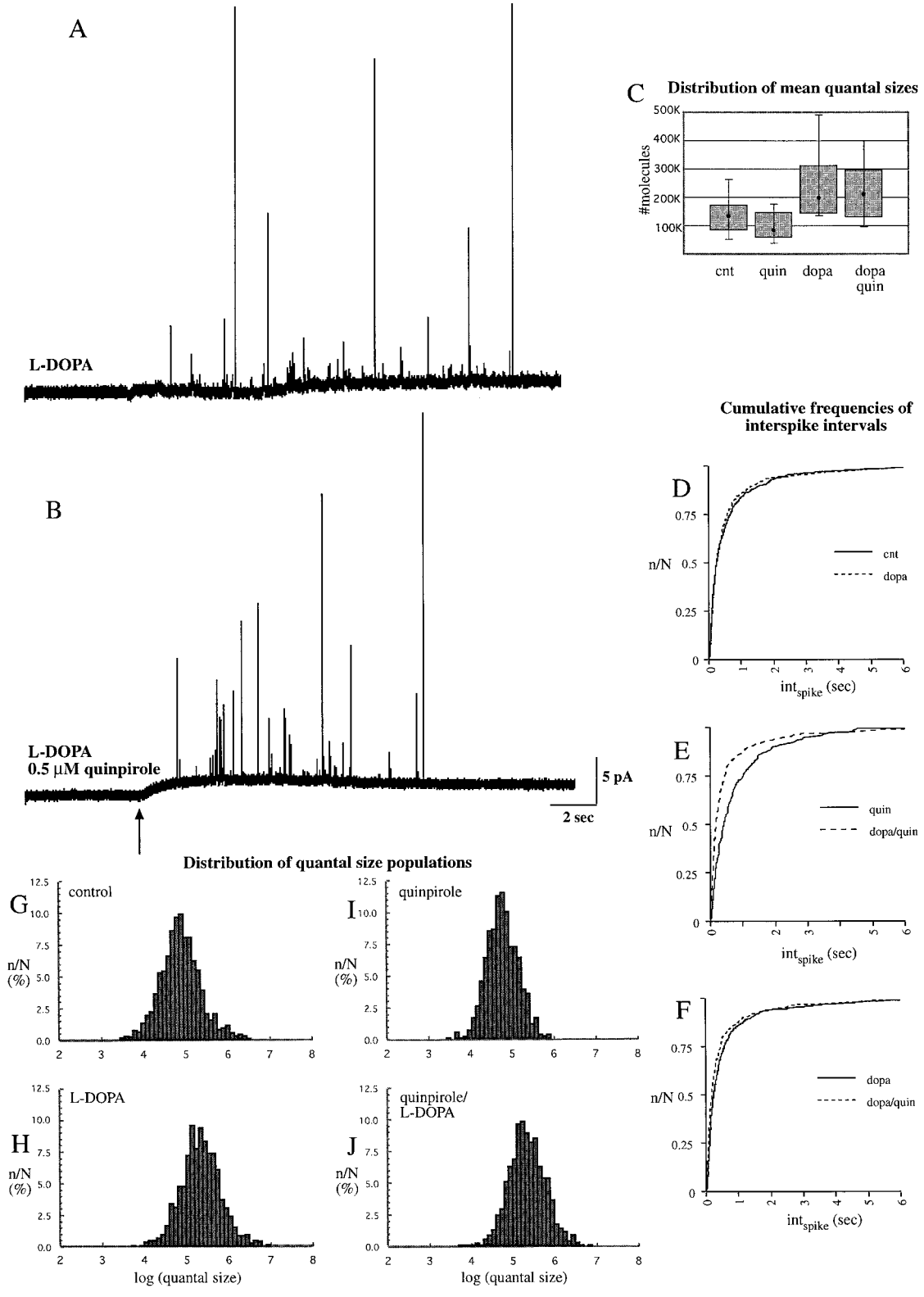

Figure 6. Effect of L-DOPA on quinpirole action. Examples of traces recorded from a cell exposed to L-DOPA (50 μM for 40 min) (*A*) and a cell that has undergone identical L-DOPA exposure in the presence of quinpirole (0.5 μM for 40 min) (*B*). Cells were stimulated by 6 sec applications of 80 mM KCl at the *arrow*. *C*, Distribution of mean quantal size values for cells that displayed more than five events within 20 sec of stimulation (controls, n = 34; quinpirole, n = 18; L-DOPA, n = 25; L-DOPA–quinpirole, n = 19). The control and quinpirole-only groups are the same as those represented in Figure 5. L-DOPA increased the mean quantal size of both control and quinpirole-treated release events to a similar degree (Table 1). *D–F*, Cumulative frequencies of interspike intervals are compared between treatments. L-DOPA blocked the effect of quinpirole on interspike intervals. *G–J*, Distribution of quantal sizes indicated as histograms of log transforms of the quantal size as in Figure 5. For comparison, the control and quinpirole-treated groups are also shown. L-DOPA shifted the frequency distribution of quantal sizes to the *right*.

et al., 1995) and is rarely invoked as a mechanism of synaptic plasticity, we and others have found that regulation of vesicular transporters (Song et al., 1997) and exposure to neurotrophic factors (Wang et al., 1995; Pothos et al., 1998) alters quantal size.

These findings may provide insights into the synaptic changes induced by psychostimulant and antipsychotic drugs. The psychostimulants cocaine and amphetamine elevate extracellular DA levels and activate mesolimbic and mesocortical D_2 autoreceptors. Therefore, psychostimulants may reduce appropriate stimulation-dependent perisynaptic DA input to postsynaptic targets by decreasing quantal size (Pothos and Sulzer, 1998) while increasing stimulation-independent overflow of extrasynaptic DA. This has important implications for the signal-to-background ratio of DA input; indeed, a precise level of DA input is required for maintenance of working memory at mesocortical connections, and it is striking that a feature of amphetamine psychosis is an impairment in working memory (Williams and Goldman-Rakic, 1995).

Classically, D_2-like autoreceptors have been classified as displaying impulse-regulating or synthesis-modulating responses. The data in this study are consistent with the possibility that both impulse-regulating and synthesis-modulating responses occur in the same cell population. D_2 autoreceptors of mesolimbic DA neurons, perhaps including D_3 autoreceptors (Meller et al., 1993; Rivet et al., 1994), inhibit firing by hyperpolarization and increasing input resistance (Grace and Bunney, 1984a) in addition to inhibiting DA synthesis. In the high-affinity state, D_2 receptors have a binding constant of ~7.5 nM (O'Dowd et al., 1994). Because background extracellular striatal DA levels are ~4 nM (Parsons and Justice, 1992), there may be significant inhibition of DA release at tonic levels, and indeed the D_2 antagonist sulpiride alone elevates DA release in awake, behaving animals (Tanaka et al., 1992). Antipsychotic drugs that act as D_2-like antagonists elevate extracellular striatal DA levels and increase DA cell firing rates after acute administration but reduce extracellular DA and firing rate after longer term exposure. A decreased quantal size would be expected to reduce stimulation-dependent diffusional overflow of DA from the release site. Because monoamine synapses provide extrasynaptic transmitter overflow (Garris et al., 1994), it is likely that alteration of quantal size plays a more important role for monoamine release than fast-acting transmitters.

In summary, these findings directly demonstrate that activation of neurotransmitter receptors alters quantal size; this apparently occurs by inhibition of transmitter synthesis. If a similar mechanism exists in neurons, alteration of quantal size by autoreceptor activation would appear to play a role in modulating synaptic plasticity.

REFERENCES

Albillos A, Dernick G, Hostmann H, Almers W, Alvarez de Toledo G, Lindau M (1997) The exocytic event in chromaffin cells revealed by patch amperometry. Nature 389:509–512.

Alvarez de Toledo G, Fernandez JM (1990) Compound versus multigranular exocytosis in peritoneal mast cells. J Gen Physiol 95:397–409.

Alvarez de Toledo G, Fernandez-Chacon R, Fernandez JM (1993) Release of secretory products during transient vesicle fusion. Nature 363:554–558.

Arbogast LA, Soares MJ, Robertson MC, Voogt JL (1993) A factor(s) from a trophoblast cell line increases tyrosine hydroxylase activity in fetal hypothalamic cell cultures. Endocrinology 133:111–120.

Bauerfeind R, Regnier-Vigouroux A, Flatmark T, Huttner WB (1993) Selective storage of acetylcholine, but not catecholamines, in neuroendocrine synaptic-like microvesicles of early endosomal origin. Neuron 11:105–121.

Bean AJ, Roth RH (1991) Extracellular dopamine and neurotensin in rat prefrontal cortex *in vivo*: effects of median forebrain bundle stimulation frequency, stimulation pattern, and dopamine autoreceptors. J Neurosci 11:2694–2702.

Bekkers JM, Richerson GB, Stevens CF (1990) Origin of variability in quantal size in cultured hippocampal neurons and hippocampal slices. Proc Natl Acad Sci USA 87:5359–5362.

Bernardini GL, Gu X, Viscardi E, German DC (1991) Amphetamine-induced and spontaneous release of dopamine from A9 and A10 cell dendrites: an *in vitro* electrophysiological study in the mouse. J Neural Transm 84:183–193.

Bohmaker K, Puza T, Goldstein M, Meller E (1989) Absence of spare autoreceptors regulating dopamine agonist inhibition of tyrosine hydroxylation in slices of rat striatum. J Pharmacol Exp Ther 248:97–103.

Booth RG, Baldessarini RJ, Marsh E, Owens CE (1994) Actions of (±)-7-hydroxy-*N*,*N*-dipropylaminotetralin (7-OH-DPAT) on dopamine synthesis in limbic and extrapyramidal regions of rat brain. Brain Res 662:283–288.

Brodie MS, Dunwiddie TV (1990) Cocaine effects in the ventral tegmental area: Evidence for an indirect dopaminergic mechanism of action. Naunyn Schmiedebergs Arch Pharmacol 342:660–665.

Bruns D, Jahn R (1995) Real-time measurement of transmitter release from single synaptic vesicles. Nature 377:62–65.

Burke NV, Han W, Li D, Takimoto K, Watkins SC, Levitan ES (1997) Neuronal peptide release is limited by secretory granule mobility. Neuron 19:1095–1102.

Cardozo DL (1993) Midbrain dopaminergic neurons from postnatal rat in long-term primary culture. Neuroscience 56:409–421.

Cass WA, Gerhardt GA (1994) Direct *in vivo* evidence that D2 dopamine receptors can modulate dopamine uptake. Neurosci Lett 176:259–263.

Cass WA, Zahniser NR (1991) Potassium channel blockers inhibit D2 dopamine, but not A1 adenosine, receptor-mediated inhibition of striatal dopamine release. J Neurochem 57:147–152.

Chen JF, Qin ZH, Szele F, Bai G, Weiss B (1991) Neuronal localization and modulation of the D2 dopamine receptor mRNA in brain of normal mice and mice lesioned with 6-hydroxydopamine. Neuropharmacology 30:927–941.

Chen TK, Luo G, Ewing AG (1994) Amperometric monitoring of stimulated catecholamine release from rat pheochromocytoma (PC12) cells at the zeptomole level. Anal Chem 66:3031–3035.

Chen G, Gavin PF, Luo G, Ewing AG (1995) Observation and quantitation of exocytosis from the cell body of a fully developed neuron in *Planorbis corneus*. J Neurosci 15:7747–7755.

Chiodo LA, Bannon MJ, Grace AA, Roth RH, Bunney BS (1984) Evidence for the absence of impulse-regulating somatodendritic and synthesis-modulating nerve terminal autoreceptors on subpopulations of mesocortical dopamine neurons. J Neurosci 12:1–16.

Chow RH, von Ruden L (1995) Electrochemical detection of secretion from single cells. In: Single-channel recording (Sakmann B, Neher E, eds), pp 245–276. New York: Plenum.

Chow RH, Von Rueden L, Neher E (1992) Delay in vesicle fusion revealed by electrochemical monitoring of single secretory events in adrenal chromaffin cells. Nature 356:60–63.

Ciolkowski EL, Maness KM, Cahill PS, Wightman RM, Evans DH, Fosset B, Amatore C (1994) Disproportionation during electrooxidation of catecholamines at carbon-fiber microelectrodes. Anal Chem 66:3611–3617.

Courtney ND, Howlett AC, Westfall TC (1991) Dopaminergic regulation of dopamine release from PC12 cells via a pertussis toxin-sensitive G-protein. Neurosci Lett 122:261–264.

Daubner SC, Lauriano C, Haycock JW, Fitzpatrick PF (1992) Site-directed mutagenesis of serine 40 of rat tyrosine hydroxylase: effects of dopamine and cAMP-dependent phosphorylation on enzyme activity. J Biol Chem 267:12639–12646.

el Mestikawy S, Hamon M (1986) Is dopamine-induced inhibition of adenylate cyclase involved in the autoreceptor-mediated negative control of tyrosine hydroxylase in striatal dopaminergic terminals? J Neurochem 47:1425–1433.

Fedele E, Andrioli GC, Ruelle A, Raiteri M (1993) Release-regulating dopamine autoreceptors in human cerebral cortex. Br J Pharmacol 110:20–22.

Finnegan JM, Pihel K, Cahill PS, Haung L, Zerby SE, Ewing AG, Kennedy RT, Wightman RM (1996) Vesicular quantal size measured

by amperometry at chromaffin, mast, pheochromocytoma, and pancreatic beta cells. J Neurochem 66:1914–1923.
Frerking M, Borges S, Wilson M (1995) Variation in GABA mini amplitude is the consequence of variation in transmitter concentration. Neuron 15:885–895.
Garris PA, Ciolkowski EL, Pastore P, Wightman RM (1994) Efflux of dopamine from the synaptic cleft in the nucleus accumbens of the rat brain. J Neurosci 14:6084–6093.
Giambalvo CT (1992) Protein kinase C and dopamine transport–1. Effects of amphetamine *in vivo*. Neuropharmacology 31:1201–1210.
Goldstein M, Harada K, Meller E, Schalling M, Hokfelt T (1990) Dopamine autoreceptors: biochemical, pharmacological, and morphological studies. Ann NY Acad Sci 604:169–175.
Grace AA, Bunney BS (1984) The control of firing pattern in nigral dopamine neurons: single spike firing. J Neurosci 4:2866–2876.
Greene LA, Sobeih MM, Teng KK (1991) Methodologies for the culture and experimental use of the PC12 rat pheochromocytoma cell line. In: Cell culture of neocortex and basal forebrain from postnatal rats (Banker G, Goslin K, eds), pp 208–226. Cambridge: MIT.
Griffith LC, Schulman H (1988) The multifunctional Ca^{2+}/calmodulin-dependent protein kinase mediates Ca^{2+}-dependent phosphorylation of tyrosine hydroxylase. J Biol Chem 263:9542–9549.
Harada K, Wu J, Haycock JW, Goldstein M (1996) Regulation of L-DOPA biosynthesis by site-specific phosphorylation of tyrosine hydroxylase in AtT-20 cells expressing wild-type and serine 40-substituted enzyme. J Neurochem 67:629–635.
Hayashi Y, Miwa S, Lee K, Koshimura K, Hamahata K, Hasewaga H, Fujiwara M, Watanabe Y (1990) Enhancement of *in vivo* tyrosine hydroxylation in the rat adrenal gland under hypoxic conditions. J Neurochem 54:1115–1121.
Haycock JW, Haycock DA (1991) Tyrosine hydroxylase in rat brain dopaminergic nerve terminals. Multiple-site phosphorylation *in vivo* and in synaptosomes. J Biol Chem 266:5650–5657.
Hetey L, Schwitzkowsky R, Ott T, Barz H (1991) Diminished synaptosomal dopamine (DA) release and DA autoreceptor supersensitivity in schizophrenia. J Neural Transm 83:25–35.
Hull EM, Bazzett TJ, Warner RK, Eaton RC, Thompson JT (1990) Dopamine receptors in the ventral tegmental area modulate male sexual behavior in rats. Brain Res 512:1–6.
Inoue K, Nakazawa K, Watano T, Ohara-Imaizumi M, Fujimori K, Takanaka A (1992) Dopamine receptor agonists and antagonists enhance ATP-activated currents. Eur J Pharmacol 215:321–324.
Johnson EA, Tsai CE, Lucci J, Harrison-Shahan Y, Azzaro AJ (1992) Dopamine D2 synthesis-modulating receptors are present in the striatum of the guinea pig. Neuropharmacology 31:95–101.
Kennedy RT, Jones SR, Wightman RM (1992) Dynamic observation of dopamine autoreceptor effects in rat striatal slices. J Neurochem 59:449–455.
Koide M, Cho AK, Howard BD (1986) Characterization of xylamine binding to proteins of PC12 pheochromocytoma cells. J Neurochem 47:1277–1285.
Krueger BK (1990) Kinetics and block of dopamine uptake in synaptosomes from rat caudate nucleus. J Neurochem 55:260–267.
Kumer SC, Vrana KE (1996) Intricate regulation of tyrosine hydroxylase activity and gene expression. J Neurochem 67:443–462.
Liu Y, Schweitzer ES, Nirenberg MJ, Pickel VM, Evans CJ, Edwards RH (1994) Preferential localization of a vesicular monoamine transporter to dense core vesicles in PC12 cells. J Cell Biol 127:1419–1433.
McElvain JS, Schenk JO (1992) Blockade of dopamine autoreceptors by haloperidol and the apparent dynamics of potassium-stimulated endogenous release of dopamine from and reuptake into striatal suspensions in the rat. Neuropharmacology 31:649–659.
Meiergerd SM, Patterson TA, Schenk JO (1993) D2 receptors may modulate the function of the striatal transporter for dopamine: kinetic evidence from studies *in vitro* and *in vivo*. J Neurochem 61:764–767.
Meller E, Bohmaker K, Goldstein M, Basham DA (1993) Evidence that striatal synthesis-inhibiting autoreceptors are dopamine D_3 receptors. Eur J Pharmacol 249:R5–R6.
Mercuri NB, Calabresi P, Bernardi G (1992) The electrophysiological actions of dopamine and dopaminergic drugs on neurons of the substantia nigra pars compacta and ventral tegmental area. Life Sci 51:711–718.
Nakanishi N, Onozawa S, Matsumoto R, Hasegawa H, Yamada S (1995) Cyclic AMP-dependent modulation of vesicular monoamine transport in pheochromocytoma cells. J Neurochem 64:600–607.
Nissbrandt H, Pileblad E, Carlsson A (1985) Evidence for dopamine release and metabolism beyond the control of nerve impulses and dopamine receptors in rat substantia nigra. J Pharm Pharmacol 37:884–889.
O'Dowd BF, Seeman P, George SR (1994) Dopamine receptors. In: Handbook of receptors and channels (Peroutka SJ, ed), pp 95–124. Boca Raton, FL: CRC.
Onali P, Olianas MC (1989) Involvement of adenylate cyclase inhibition in dopamine autoreceptor regulation of tyrosine hydroxylase in rat nucleus accumbens. Neurosci Lett 17:91–96.
Palij P, Bull DR, Sheehan MJ, Millar J, Stamford J, Kruk ZL, Humphrey PP (1990) Presynaptic regulation of dopamine release in corpus striatum monitored *in vitro* in real time by fast cyclic voltammetry. Brain Res 509:172–174.
Parsons LH, Justice Jr JB (1992) Extracellular concentration and *in vivo* recovery of dopamine in the nucleus accumbens using microdialysis. J Neurochem 58:212–218.
Pitts DK, Kelland MD, Freeman AS, Chiodo LA (1993) Repeated amphetamine administration: Role of forebrain in reduced responsiveness of nigrostriatal dopamine neurons to dopamine agonists. J Pharmacol Exp Ther 264:616–621.
Pothos EN, Sulzer D (1998) Modulation of quantal dopamine release by psychostimulants. Adv Pharmacol 42:198–202.
Pothos EN, Desmond M, Sulzer D (1996) L-3,4-Dihydroxyphenylalanine increases the quantal size of exocytic dopamine release *in vitro*. J Neurochem 66:629–636.
Pothos EN, Davila V, Sulzer D (1998) Presynaptic recording of quanta from midbrain dopamine neurons and modulation of the quantal size. J Neurosci 18:4106–4118.
Ramachandran B, Houben K, Rozenberg YY, Haigh JR, Varpetian A, Howard BD (1993) Differential expression of transporters for norepinephrine and glutamate in wild type, variant, and WNT1-expressing PC12 cells. J Biol Chem 268:23891–23897.
Rivet JM, Audinot V, Gobert A, Peglion JL, Millan MJ (1994) Modulation of mesolimbic dopamine release by the selective dopamine D3 receptor antagonist, (+)-S 14297. Eur J Pharmacol 265:175–177.
Salah RS, Kuhn DM, Galloway MP (1989) Dopamine autoreceptors modulate the phosphorylation of tyrosine hydroxylase in rat striatal slices. J Neurochem 52:1517–1522.
Saller CF, Salama AI (1984) Dopamine synthesis in synaptosomes: relation of autoreceptor functioning to pH, membrane depolarization, and intrasynaptosomal dopamine content. J Neurochem 43:675–688.
Santiago M, Westerink BHC (1991) Characterization and pharmacological responsiveness of dopamine release recorded by microdialysis in the substantia nigra of conscious rats. J Neurochem 57:738–747.
Schroeder TJ, Jankowski JA, Kawagoe KT, Wightman RM, Lefrou C, Amatore C (1992) Analysis of diffusional broadening of vesicular packets of catecholamines released from biological cells during exocytosis. Anal Chem 64:3077–3083.
Schubert D, LaCorbiere M, Klier FG, Steinbach JH (1980) The modulation of neurotransmitter synthesis by steroid hormones and insulin. Brain Res 190:67–79.
Sesack SR, Aoki C, Pickel VM (1994) Ultrastructural localization of D2 receptor-like immunoreactivity in midbrain dopamine neurons and their striatal targets. J Neurosci 14:88–106.
Seutin V, Verbanck P, Massotte L, Dresse A (1991) Acute amphetamine-induced subsensitivity of A10 dopamine autoreceptors *in vitro*. Brain Res 558:141–144.
Silvia CP, King GR, Lee TH, Zy X, Caron MG, Ellinwood EH (1994) Intranigral administration of D2 dopamine receptor antisense oligodeoxynucleotides establishes a role for nigrostriatal D2 autoreceptors in the motor actions of cocaine. Mol Pharmacol 46:51–57.
Sonders MS, Zhu SJ, Zahniser NR, Kavanaugh MP, Amara SG (1997) Multiple ionic conductances of the human dopamine transporter: the actions of dopamine and psychostimulants. J Neurosci 17:960–974.
Song HJ, Ming GL, Fon E, Bellocchio E, Edwards RH, Poo MM (1997) Expression of a putative vesicular acetylcholine transporter facilitates quantal transmitter packaging. Neuron 18:815–826.
Steyer JA, Horstmann H, Almers W (1997) Transport, docking and exocytosis of single secretory granules in live chromaffin cells. Nature 474–478.
Strait KA, Kuczenski R (1986) Dopamine autoreceptor regulation of the kinetic state of striatal tyrosine hydroxylase. Mol Pharmacol 29:561–569.
Sulzer D, Chen TK, Lau YY, Kristensen H, Rayport S, Ewing A (1995)

Amphetamine redistributes dopamine from synaptic vesicles to the cytosol and promotes reverse transport. J Neurosci 15:4102–4108.
Surmeier DJ, Song WJ, Yan Z (1996) Coordinated expression of dopamine receptors in neostriatal medium spiny neurons. J Neurosci 16:6579–6591.
Tanaka T, Vincent SR, Nomikos GG, Fibiger HC (1992) Effect of quinine on autoreceptor-regulated dopamine release in the rat striatum. J Neurochem 59:1640–1645.
Tepper JM, Groves PM (1990) *In vivo* electrophysiology of central nervous system terminal autoreceptors. Ann NY Acad Sci 604:470–487.
Thompson SM, Capogna M, Scanziani M (1993) Presynaptic inhibition in the hippocampus. Trends Neurosci 16:222–227.
Timmerman W, De Vries JB, Westerink BHC (1990) Effects of D-2 agonists on the release of dopamine: localization of the mechanism of action. Naunyn Schmiedebergs Arch Pharmacol 342:650–654.
Tissari AH, Lillgèls MS (1993) Reduction of dopamine synthesis inhibition by dopamine autoreceptor activation in striatal synaptosomes with *in vivo* reserpine administration. J Neurochem 61:231–238.
Van der Kloot W (1991) The regulation of quantal size. Prog Neurobiol 36:93–130.
Wang T, Xie K, Lu B (1995) Neurotrophins promote maturation of developing neuromuscular synapses. J Neurosci 15:4796–4805.
Westerink BHC, De Boer P, Timmerman W, De Vries JB (1990) *In vivo* evidence for the existence of autoreceptors on dopaminergic, serotonergic, and cholinergic neurons in the brain. Ann NY Acad Sci 604:492–504.
Wieczorek WJ, Kruk ZL (1994) A quantitative comparison on the effects of benztropine, cocaine and nomifensine on electrically evoked dopamine overflow and rate of re-uptake in the caudate putamen and nucleus accumbens in the rat brain slice. Brain Res 657:42–50.
Wightman RM, Jankowski JA, Kennedy RT, Kawagoe KT, Schroeder TJ, Leszczyszyn DJ, Near JA, Diliberto Jr EJ, Viveros OH (1991) Temporally resolved catecholamine spikes correspond to single vesicle release from individual chromaffin cells. Proc Natl Acad Sci USA 88:10754–10758.
Williams GW, Goldman-Rakic PS (1995) Modulation of memory fields by dopamine D1 receptors in prefrontal cortex. Nature 376:572–575.
Xue ZY, Caron MG, Ellinwood EH (1994) Intranigral administration of D2 dopamine receptor antisense oligodeoxynucleotides establishes a role for nigrostriatal D2 autoreceptors in the motor actions of cocaine. Mol Pharmacol 46:51–57.
Yamada S, Yokoo H, Nishi S (1991) Changes in sensitivity of dopamine autoreceptors in rat striatum after subchronic treatment with methamphetamine. Eur J Pharmacol 205:43–47.
Zhang H, Lee TH, Ellinwood Jr EH (1992) The progressive changes of neuronal activities of the nigral dopaminergic neurons upon withdrawal from continuous infusion of cocaine. Brain Res 594:315–318.
Zhou Z, Misler S (1995) Detection of quantal release of catecholamines from developing neurons in culture by amperometry. Biophys J 39A:398.
Zhou Z, Misler S (1996) Amperometric detection of quantal secretion from rat pancreatic beta-cells. J Biol Chem 270:270–277.
Zhou R, Luo G, Ewing AG (1994) Direct observation of the effect of autoreceptors on stimulated release of catecholamines from adrenal cells. J Neurosci 14:2402–2407.
Zhu HJ, Sonders MS, Amara SG, Kavanaugh MP, Zahniser NR (1995) Uptake of dopamine by the cloned human dopamine transporter is voltage-dependent in *Xenopus* oocytes. Soc Neurosci Abstr 21:375.