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Articles

Voltammetric and Pharmacological Characterization of Dopamine Release from Single Exocytotic Events at Rat Pheochromocytoma (PC12) Cells

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Although rat pheochromocytoma (PC12) neurotransmitter storage vesicles are known to contain a variety of neurotransmitters including catecholamines, there is little evidence that the molecular species detected during amperometric monitoring of exocytosis is a catecholamine. Rather, as these are catecholamine-containing cells, one assumes catecholamines are released. Additionally, although the total amount of transmitter released can be quantified, it has been extremely difficult to evaluate the conentration at the point of release for each exocytosis event. Interpreting voltammograms obtained in the attoliter volume affected between the electrode and the cell and defined by the size of the exocytosis pore during exocytosis is an extreme analytical challenge. Here we use voltammetry of $\sim 10^{-19}$ mol released from individual exocytosis events to identify, along with pharmacological evidence, the released compound at PC12 cells as a catecholamine, most likely dopamine. The area of the electrode at which oxidation occurs following an exocytosis event is proportional to the temporal delay prior to acquisition of a voltammogram. This model allows determination of relative concentrations from individual release events and has been used to examine events at control cells and cells incubated with the dopamine precursor, L-3,4-dihydroxyphenylalanine (L-DOPA). Exposure to L-DOPA (100 μM for 1 h) results in 145 detectable events for 11 cells compared to 77 events for 29 control cells, clearly indicating that vesicles can be "loaded" with dopamine. However, the concentrations measured at the electrode surface provide similar distributions for both L-DOPA-treated and control cells. Cyclic voltammetric measurements of relative concentration for zeptomole levels of transmitter in attoliter volumes provide evidence that loading vesicles by increased transmitter synthesis does not lead to elevated concentrations at individual release sites.

The exocytotic release of chemical messengers into the extracellular environment is one of the defining mechanisms of intercellular communication and is a major research theme.[1,2] Unraveling the mechanisms behind this process is challenging, however, since the release events occur within a few milliseconds.[3,4] This transient nature of exocytosis demands a rapid detection scheme that is both sensitive and selective if release events are to be monitored in real-time. This analytical challenge has been met in many respects with the advent of carbon fiber microelectrodes.[5] The rapid response times (μs) of microelectrodes make them an ideal tool for monitoring the dynamic process of exocytosis, and their intrinsic nature provides selectivity in that several commonly secreted chemical messengers are easily oxidized. Furthermore, the ability to fabricate microelectrodes routinely to micrometer dimensions allows measurements to be confined to very small spaces, such as the surface of a single cell[6−8] or a varicosity on a developing neurite.[9,10]

Constant potential amperometry is most often employed for experiments of this nature as it offers the highest sensitivity and fastest response time to changes in concentration. Additionally, quantitation of amperometric data is straightforward since the total charge passed at the electrode surface is directly proportional to the amount of analyte detected. Amperometry with microelectrodes has allowed single exocytotic release events to be studied

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in significant detail. Several research groups are currently using this detection scheme to investigate the amplitude, temporal aspects, and location of individual release events from single cells. More recently, this technique has been extended to features that occur directly before the more prominent release events, such as the leakage of transmitter through the fusion pore complex.[11,12] Reviews that offer a more thorough discussion of the subject are available.[13−16]

A drawback of amperometric monitoring at single cells is that chemical identification must be sacrificed for temporal resolution. This is a concern when one considers that numerous other molecules are present in synaptic vesicles.[17] Voltammetry, on the other hand, can be used to discriminate between molecules with different oxidation/reduction properties. Several neurotransmitters can be identified in this manner on the basis of characteristic voltammograms obtained by fast-scan rate cyclic voltammetry.[18] In a landmark paper, Wightman and co-workers demonstrated that cyclic voltammetry can be used to identify catecholamines released from single bovine adrenal chromaffin cell vesicles.[7] Cyclic voltammetry has also been used to differentiate between epinephrine and norepinephrine released from these bovine adrenal cell vesicles[19] and to provide direct evidence of histamine and serotonin cosecretion from single rat peritoneal mast cell vesicles.[20]

We are interested in understanding exocytosis from rat pheochromocytoma (PC12) cells since this clonal cell line exhibits many of the physiological properties of sympathetic ganglion neurons and has frequently been used as a model for the developing sympathetic nerve.[21,22] The chemical nature and small size ($r = 74$ nm) of catecholamine-secreting vesicles in PC12 cells suggest that they may be valid analogues of brain synaptic vesicles, and their study should improve the understanding of exocytosis at the synapse.[23] The average release event from PC12 cells has been calculated at 110 000 molecules, or 180 zmol, using amperometry.[24]

Despite the chemical characterization of transmitter release from this clonal cell line, there has not yet been direct evidence that the species detected during amperometric monitoring of exocytosis is catecholamine. The current transients observed have been tentatively assigned to the release of catecholamine since dopamine (DA) and norepinephrine (NE) are the only known electrochemically active molecules found at significant levels in PC12 cell cultures.[21−23] In this paper, we use amperometric and voltammetric methods at the zeptomole level to provide direct evidence that the species monitored during electrochemical detection of exocytosis from PC12 cells is catecholamine. The amplitude and frequency of amperometric events in the presence of the monoamine vesicle depleting agent, reserpine (1 μM), have been monitored to provide indirect evidence that the detected species is catecholamine. More conclusively, cyclic voltammetry at 300 V/s has been used to obtain complete voltammograms within the expected time frame of a single release event, and these voltammograms resemble those for the catecholamines. At this level, we cannot yet discriminate between DA and NE. Cyclic voltammetry also provides the means to estimate the concentration of released substance during each event. Using the DA precursor, L-3,4-dihydroxyphenylalanine (L-DOPA), we have examined the relative concentrations for exocytosis events and correlated this information with the number of events producing observable concentrations to define the effects of L-DOPA on concentration in the vesicle.

EXPERIMENTAL SECTION

Reagents and Solutions. All chemicals were reagent grade and used without further purification. Physiological saline consisted of 4.2 mM KCl, 150 mM NaCl, 2 mM $CaCl_2$, 0.7 mM $MgCl_2$, 1 mM NaH_2PO_4, and 10 mM HEPES. Physiological saline with elevated K^+ consisted of 80 mM KCl, 50 mM NaCl, 2 mM $CaCl_2$, 0.7 mM $MgCl_2$, 1 mM NaH_2PO_4, and 10 mM HEPES. All solutions were prepared in distilled water (2×) and adjusted to pH 7.4. DA standards were prepared by serial dilution in deoxygenated physiological saline.

Cell Culture. PC12 cells were obtained from Lloyd Greene (Columbia University) and maintained as previously described[25] in phenol red-free RPMI-1640 media (Gibco, Grand Island, NY) supplemented with 10% heat-inactivated equine serum, 5% fetal bovine serum (Hyclone Laboratories, Logan, UT), and 0.4% penicillin streptomycin solution (Sigma Chemical Co., St. Louis, MO) in a 7% CO_2, 100% humidity atmosphere at 37 °C. The cells were grown on mouse collagen (type IV, 0.5 μg/cm^2, Collaborative Biomedical Products, Bedford, MA) coated cell culture dishes (60 mm) and were subcultured every 7−9 days. The medium was replaced every 2−3 days throughout the lifetime of all cultures.

Electrochemical Measurements. Carbon fiber microelectrodes were prepared by aspirating single 5-μm-diameter carbon fibers (Amoco, Greenville, SC) into 1.2 mm × 0.68 mm glass capillaries (A-M Systems, Everett, WA) and pulling the capillaries to the dimensions of the fiber with a vertical puller (Ealing, Harvard Apparatus, Edenbridge, KY). The fibers were trimmed to ∼1 cm beyond the glass-fiber junction, dipped into epoxy (Epo-Tek, Epoxy Technology, Billerica, MA) for 60 s, and cured at 100 °C for 90 min. The electrodes were back-filled with colloidal graphite (Energy Beam Sciences, Agawam, MA), and Nichrome wires were inserted for electrical contact. Extending fibers were trimmed further back to the glass-fiber junction to expose a fresh carbon surface. Electrode tips were beveled at a 45° angle on a micropipet beveler (World Precision Instruments, New Haven, CT) with diamond paste (0.25-μm diameter, Buehler, Lake Bluff,

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IL) for 1−2 min and sonicated for 20−30 s in a 75% methanol solution to clean the electroactive surface. Prior to use, electrode tips were examined under a microscope and tested in a freshly prepared 0.1 mM DA solution. Electrodes with irregular bevels and/or unstable responses were discarded.

Cyclic voltammetry was performed with an EI-400 potentiostat (Ensman Instrumentation, Bloomington, IN) in the two-electrode mode. Sodium-saturated calomel electrodes (SSCE) were constructed in-house and used as reference electrodes for all measurements. Cyclic voltammetry was employed at a scan rate of 300 V/s using a triangle wave form that was ramped between −0.5 and +1.0 V. There was no delay time between successive scans. Data were acquired at 50 kHz (20 μs/point) using commercially available software (Axosope 1.1.1, Axon Instruments, Foster City, CA). The current response was filtered at 2 kHz and digitized with a 12-bit analog to digital converter (Digidata 1200, Axon Instruments) interfaced to a Gateway 2000 486/33C PC computer. Prior to all measurements, the current response of the electrode was allowed to equilibrate for at least 10 min.

Electrodes were calibrated in a flow injection apparatus similar to that previously described.[26] Briefly, the electrode tip was positioned a small distance inside the exit tube of a loop injector (model 7010, Rheodyne, Inc., Cotati, CA). Physiological saline was pumped through the flow system with a syringe pump (Pump 22, Harvard Apparatus, South Natick, MA) at a rate of 1.0 mL/min, and a series of DA standards were injected manually into the flowing stream at regular intervals. Cyclic voltammograms were collected before, during, and after the injection of standard solution into the flowing buffer.

Amperometry was performed with an Axopatch 200B potentiostat (Axon Instruments) at an applied potential of +700 mV. In this case, the current response was filtered at 10 kHz and the analog to digital converter was interfaced to a Power Macintosh 7200 computer.

Single-Cell Experiments. All PC12 cell experiments were performed 5−10 days after plating. Prior to each experiment, the cells were rinsed three times and culture medium was replaced with 5 mL of warm (37 °C) physiological saline. For L-DOPA experiments, cells were incubated in medium containing 100 μM L-DOPA (Sigma) for 1 h and rinsed three times prior to experimentation. For reserpine experiments, the existing physiological saline was adjusted to 1 μM reserpine (Sigma) 2 min after the first stimulation. Physiological saline with elevated K^+ (80 mM) was used as the membrane-depolarizing stimulant for all single-cell experiments. Fast green dye (0.1 wt %) was used as a visual aid for some experiments.

All cell experiments were performed on the stage of an inverted microscope (IM-35, Carl Zeiss, Thornwood, NY) at room temperature. The working electrode was pushed gently against the target cell with a piezomicropositioner (PCS-750/1000, Burleigh Instruments). A micropipet (1 μm tip), filled with stimulant, was positioned near the bottom of the dish ~50 μm from the target cell by a micropositioner (Zeiss, Germany). Stimulating agents were delivered by a 5-s pressure (6−8 psi) injection (Picospritzer II, General Valve, Fairfield, NJ). Total injection volume was not a concern and therefore not measured in this experiment.

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Data Treatment. Locally written programs and LabVIEW software (National Instruments Corp., Austin, TX) were used for analysis of voltammetric data. To speed the analysis process, data points were collected for a maximum of 30 s for each cell tested. Cyclic voltammograms of DA were identified by constructing a current−time trace of the average current collected in a 60-mV window near the peak oxidation potential expected for this species. Final cyclic voltammograms were revealed by digital subtraction of background collected immediately prior to DA signal. All voltammograms shown in this work were background subtracted and smoothed (9-point moving window) with the peak current of the anodic wave being used for calculation of concentration. Calibration plots were prepared from regression of the standard peak height vs concentration. All errors are reported as relative standard deviation (RSD).

Locally written programs and Superscope software (GWI Instruments, Medford, MA) were used for analysis of amperometric data. Peaks were identified if their rising slope was greater than a noise threshold 4.5 × rms noise. The number of molecules oxidized at the electrode surface was determined by the relation $N = Q/nF$, where Q is the charge of the peak, n is the number of electrons transferred ($n = 2$ for catecholamines), N is the number of moles, and F is Faraday's constant (96 485 C/equiv).

RESULTS AND DISCUSSION

Amperometry in the Presence of Reserpine: Pharmacological Evidence of Released Catecholamine. Amperometric monitoring of exocytosis has yielded a wealth of information regarding amplitude, temporal aspects, and location of individual exocytotic release events. However, with amperometry, molecular identification is sacrificed for temporal resolution and sensitivity. Although amperometry offers no direct means of identification, its ability to resolve individual release events can be used in conjunction with pharmacological manipulations to provide indirect evidence regarding the nature of the detected species. We demonstrate this idea by combining amperometry with application of reserpine, an inhibitor of the vesicular monoamine transporter that displaces catecholamine from neurotransmitter vesicles. A series of current transients, representing individual release events, are shown in Figure 1 for two PC12 cells monitored with amperometry. Initial conditions are identical for both cells. However, 2 min after the first K^+ stimulation, the medium surrounding the cell shown in the second column was adjusted to 1 μM reserpine. Repetitive stimulations at 5, 7, 12, 17, and 22 min show that the frequency and amplitude of individual events diminish rapidly for the cell incubated with reserpine. Total area under all current transients has been measured and compared (Figure 2) for all control cells ($n = 3$) and reserpine-treated cells ($n = 3$). When PC12 cells are incubated with 1 μM reserpine, total release per stimulation diminishes to half its initial value after ~10 min and declines steadily throughout the remainder of the experiment. Moreover, while >80% of the cells typically display amperometric events following the first high K^+ stimulation under these conditions, we observed amperometric events from only 3 cells exposed to reserpine for 35−61 min (out of 16 cells total). These data provide biological evidence that the substance released is a catecholamine. However, physical evidence through a selective analysis technique is still lacking. Hence, we have

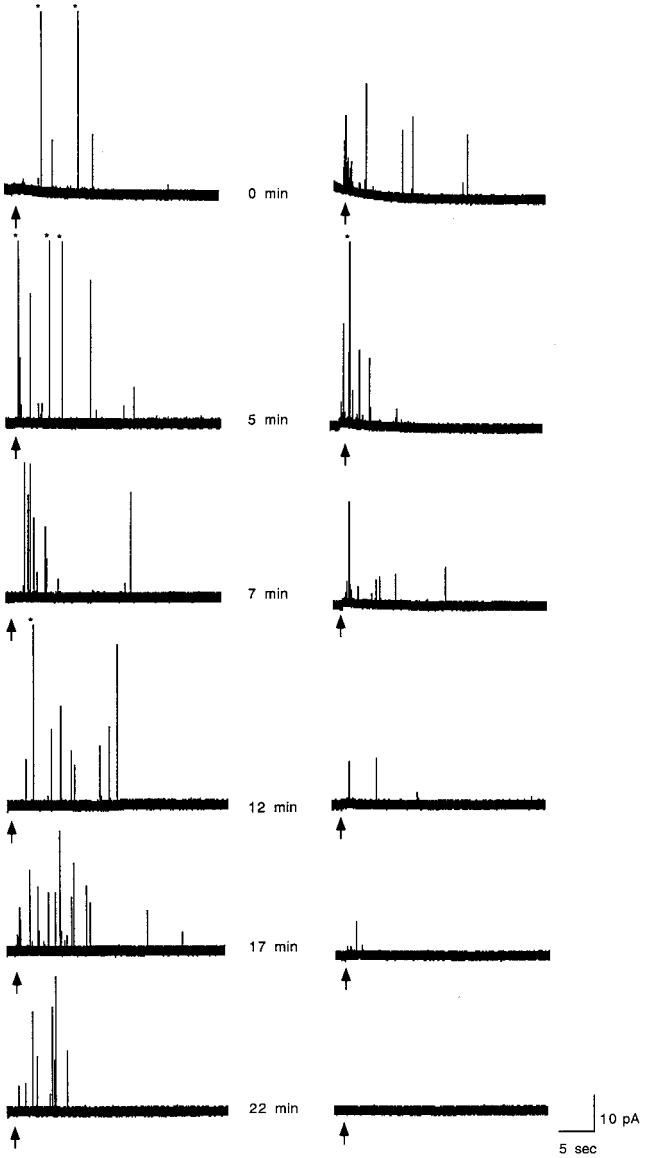

Figure 1. Effect of reserpine on quantal release from PC12 cells. In the first column, a single PC12 cell was repeatedly stimulated with 80 mM KCl for 3 s at the arrows; amperometric spikes were elicited in each case. In the second column, a different cell was treated identically except that, at 2 min following the first stimulation, 1 μM reserpine was added to the medium. Subsequent stimulations indicate that quantal release is abolished by reserpine.

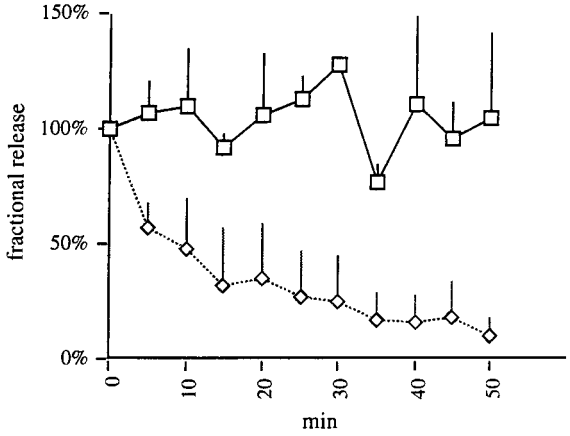

Figure 2. Effect of reserpine on total release. Experiments were conducted as in Figure 1. For each stimulation, the molecules released in all amperometric events were summed. The data points indicate fractional release of the initial stimulation (0 min). The initial stimulations released $(4.2 \pm 0.8) \times 10^6$ molecules. Release from the control cells (solid line, rectangles) was stable over successive stimulations through the last time point tested (105% of initial levels, 50 min). Release from reserpine-treated cells (broken line, diamonds) was 48% inhibited at 10 min; at 50 min, only 10% of the initial level was released. Bars indicate SEMs. $n = 3$ for each group.

pursued the use of fast-scan rate cyclic voltammetry to try to capture single exocytosis events from PC12 cells.

Cyclic Voltammetry during Stimulated Exocytosis. Voltammetric measurements of exocytosis at PC12 cells is extremely challenging to obtain since the amount of transmitter released is only at zeptomole levels and the events occur on the millisecond time scale. Furthermore, these events, although elicited by chemical stimulation, do not occur at precise times. Cyclic voltammetry was performed at 300 V/s, employing a wave form that is continuously ramped between −0.5 and +1.0 V, while cells are stimulated with elevated K^+ solution. At this rate, a complete voltammogram can be collected in 10 ms and should theoretically be able to capture single events as they occur in real-time, if the ensuing concentration is within the detection limit. A characteristic of cyclic voltammetry is the presence of a high background current at fast-scan rates. Fortunately, this nonfaradaic background current is very stable and can be digitally subtracted from the faradaic current to yield analytically useful data.[18] To determine which scans (−0.5 to 1.0 V) have increased faradaic signal owing to release, an average of 10 data points collected within a 60-mV window of the peak oxidation potential of DA is continuously monitored and examined (Figure 3A). Focusing in further (Figure 3B), it can be seen that individual current transients rise very sharply and decay very rapidly. In most cases, faradaic current is only during a single scan. Removal of background signal (average of scans 687−690) from the faradaic response (scan 693) reveals a voltammogram (Figure 3C) that has the characteristic features of DA in standard solution. For comparison, Figure 3D shows a background-subtracted voltammogram of a standard 25 μM DA solution collected from the flow system described earlier. A total of 77 voltammograms have been detected from stimulated exocytosis of 29 PC12 cells. The relative infrequency of detectable events is apparently due to the low levels of transmitter released from individual vesicles in PC12 cells (vide infra).

Cyclic Voltammetry after Exposure to L-DOPA. Similar to the idea that reserpine decreases catecholamine levels, pharmacological manipulation with the DA precursor L-DOPA should increase vesicular accumulation. Previous amperometric measurements have shown dramatic increases in the magnitude of individual current transients when PC12 cells are incubated in L-DOPA prior to experimentation.[27] Fast-scan rate cyclic voltammetry has been performed at a number of PC12 cells that have been exposed to L-DOPA (100 μM for 1 h) and data were analyzed as described above. A representative voltammogram (Figure 4C) collected in this manner shows the characteristics of DA in standard solution (Figure 4D). The frequency of detectable events increases considerably (Figure 4A). A total of 145 events were

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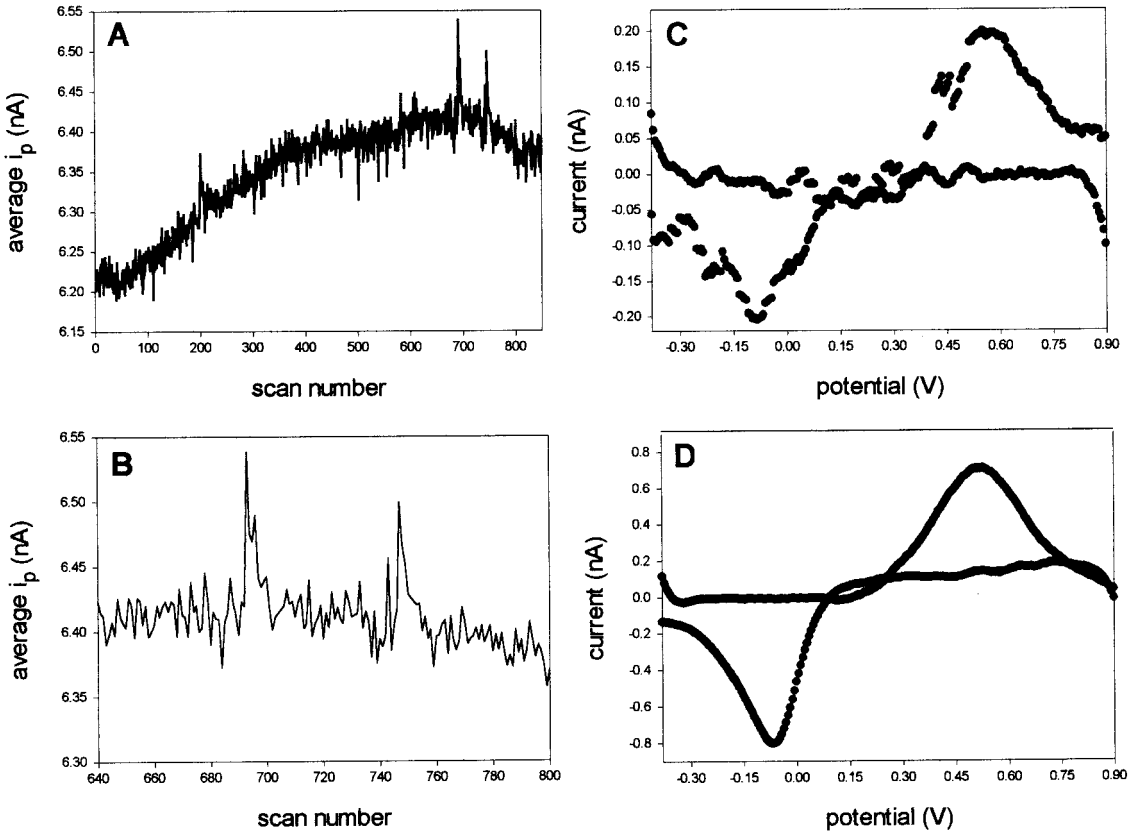

Figure 3. Fast-scan rate cyclic voltammetry data collected during stimulated exocytosis of a single PC12 cell. (A) Average current within a 60-mV window of the peak oxidation potential of DA. Individual current spikes represent faradaic current. (B) Enlargement of the region between scans 600 and 800 showing two current transients used to generate voltammograms. (C) Background-subtracted voltammogram generated by subtracting the average signal of scans 687−690 from the signal of scan 693. (D) Background-subtracted voltammograms of a standard 25 μM DA solution.

detected from only 11 cells when preincubated with L-DOPA. It appears that L-DOPA "loads" vesicles with catecholamine, thus increasing the number of vesicles for which release can be detected with voltammetry. Additionally, each event has a larger half-width representing a longer time of release when compared to cells that are not incubated with L-DOPA. Thus, the faradaic signal is detected over a larger number of scans for each individual event (Figure 4B). Therefore, we conclude that L-DOPA increases the amount of catecholamine in at least a subset of PC12 vesicles.

Evaluation of Concentration: Electrode Calibration and Histogram Construction. A plot of peak current (i_p) vs standard DA concentration has been prepared for each electrode used in the voltammetry experiments to evaluate the concentration of measured exocytotic events. Calibration plots are linear ($r^2 >$ 0.95) for concentrations up to 15 μM DA when the peak current of the anodic wave is used as the reference point. An estimate of the concentration during exocytosis can be obtained by comparison of the peak current for voltammograms obtained for individual exocytosis events to the calibration plot. These estimates are lower than expected for vesicle concentrations as the released catecholamine only accesses a very small area of the electrode, whereas the calibration is carried out in a solution accessing the entire electrode surface (vide infra). It is important to note that we do not expect to measure the absolute concentration of vesicular species when performing an experiment of this nature. Rather, we expect to measure an estimated fraction of the true vesicular concentration due to differences in the area of electrode used for oxidation/reduction in standard solution vs the single-vesicle case (vide infra). However, relative concentrations can be evaluated.

Modeling Voltammetry To Obtain Approximate Concentrations of Released Neurotransmitter. Measurement of absolute concentrations released from a single vesicle with voltammetry is problematic, but an estimated fraction of the concentration can be accomplished. A simple model, outlined in pictorial form in Figure 5, has been developed to explain this concept. Consider first the general cyclic voltammetry equation for peak current:

$$i_p = (2.69 \times 10^5) n^{3/2} A D_o^{1/2} \upsilon^{1/2} C_o^* \qquad (1)$$

where i_p is peak current, n is number of electrons transferred, A is electrode area of oxidation/reduction, D is diffusion coefficient of the analyte, υ is scan rate, and C is concentration of analyte. It is evident in Figure 5 that the area of the electrode used for oxidation/reduction will be significantly smaller for substances released from a single vesicle compared to a standard solution of the same substance. Using an average of 74 nm for vesicle radius[23] and an average electroactive area of 28 μm^2 for a 5-μm fiber beveled at a 45° angle, and assuming total exocytosis and complete recovery of vesicle contents, eq 1 would predict that the apparent average concentration measured from exocytosis of a single vesicle is ~0.061% of its true concentration in standard

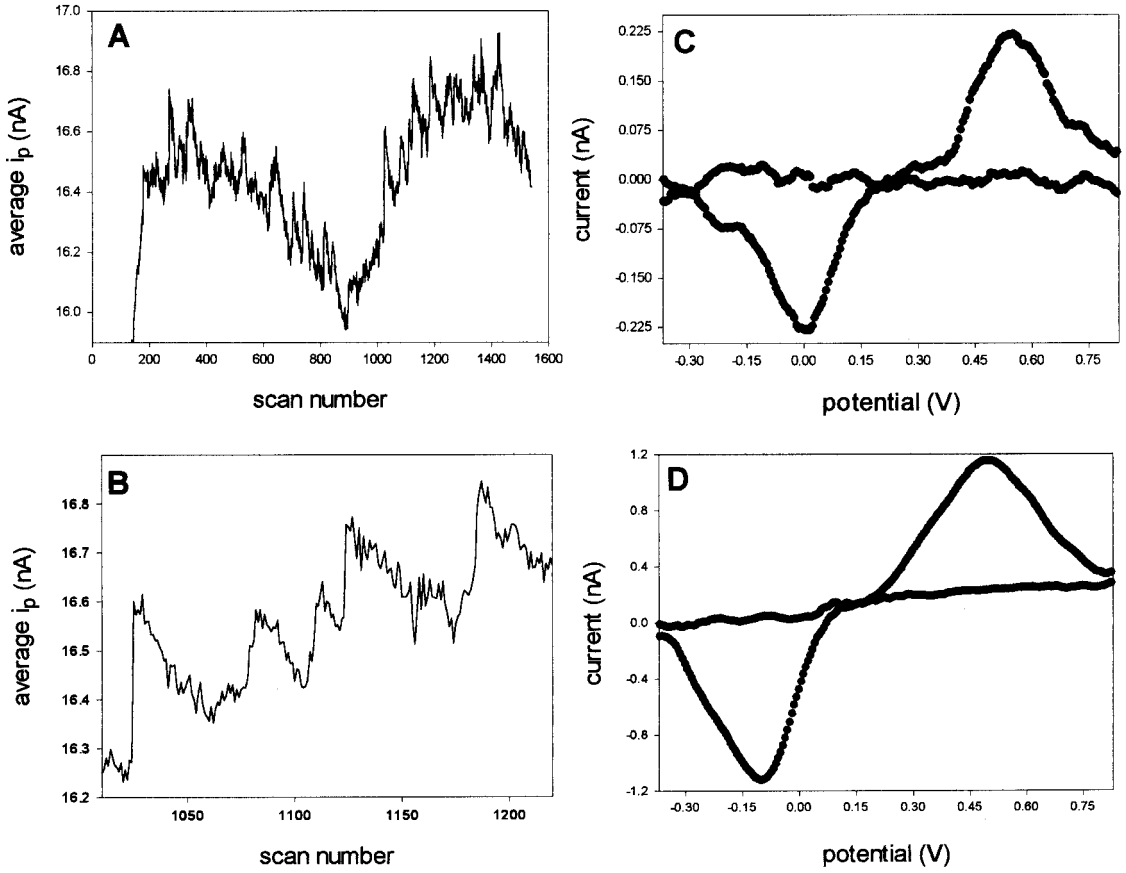

Figure 4. Fast-scan rate cyclic voltammetry data collected during stimulated exocytosis of a single PC12 cell after exposure to 100 μM L-DOPA for 1 h. (A) Average current within a 60-mV window of the peak oxidation potential of DA. Individual current spikes represent faradaic current. (B) Enlargement of the region between scans 1010 and 1225 showing multiple current transients used to generate voltammograms. (C) Background-subtracted voltammogram generated by subtracting the average signal of scans 1020−1023 from the signal of scans 1025−1026. (D) Background-subtracted voltammogram of a standard 25 μM DA solution.

solution. Since vesicles in PC12 cells are believed to have a catecholamine concentration of ~0.11 M,[24] this translates to a maximum of ~67 μM. The small size of PC12 cell vesicles guarantees, however, that significant dilution will occur before the substance diffuses to the electrode surface. During the exocytotic event, the transmitter diffuses into the space between the cell and the electrode leading to dilution. Thus, the observed concentration reflects that in the gap between the cell and the electrode and is only proportional to that in the vesicle. The average concentration measured at control cells is 3.35 ± 0.24 μM (n = 77), which reflects this dilution. An understanding of this simple model illustrates the difficulty of detecting exocytotic events of this magnitude using cyclic voltammetry. Although this model suggests that absolute intravesicular concentrations cannot be measured with voltammetry, relative concentrations can be evaluated and qualitative analysis can still be performed.

Another aspect to the model of voltammetry of single exocytosis events is the delay between the exocytosis event and the voltammogram. The exocytosis events occur over a period of only a few milliseconds. At a scan rate of 300 V/s and a range of 1.5 V, each voltammogram requires 10 ms. Thus, an important consideration is the effect of diffusion following the event on the affected electrode area and the concentration of neurotransmitter at any point in the voltammogram. This turns out to be simpler than originally expected as the solution between the electrode and cell membrane can be modeled as a cylinder. As the released neurotransmitter diffuses, the concentration in the cylinder (volume $\pi r^2 l$) is inversely proportional to the area of the electrode at which oxidation occurs (area πr^2). Thus, estimates of concentration based on the original electrode area that is exposed to the released neurotransmitter provide useful quantitation of release for comparison between cell types and conditions without significant contributions from the time of release during individual voltammograms. This allows comparison of the relative concentrations of catecholamine for control vs L-DOPA-treated PC12 cells.

Histograms of frequency vs apparent concentration have been prepared for voltammograms collected from cells with and without L-DOPA exposure (Figure 6). Interestingly, the two data sets are very similar. Although there are a few more highly concentrated events detected after L-DOPA exposure, the bulk of the data for each set appear to be distributed over a similar concentration range. The average concentration measured in this experiment is 3.47 ± 0.17 μM for events from L-DOPA-exposed cells, as compared to 3.35 ± 0.24 μM (reported above) for control cells. These results support the idea that vesicles might increase in size as catecholamine content increases or perhaps that the rate of catecholamine efflux is not primarily dependent on the intravesicular concentration.

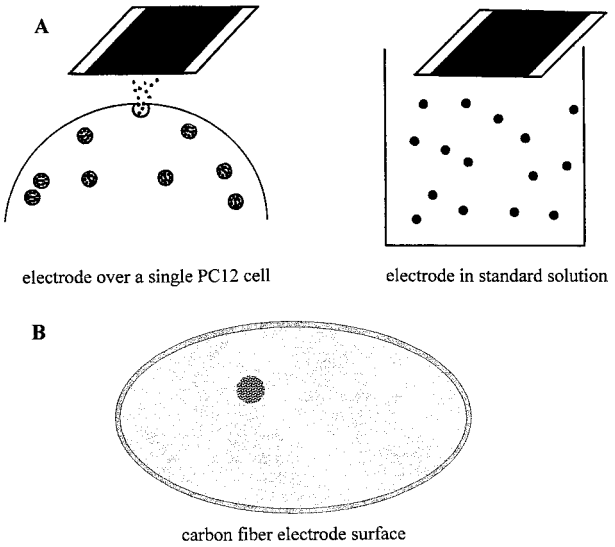

Figure 5. Simple model of the concentration of vesicular events as determined by fast-scan rate cyclic voltammetry. (A) This pictorial demonstrates that the area of the electrode used for oxidation/reduction of the DA species will be very different for the cellular case vs that in standard solution. (B) This is a head-on view depicting the difference in electrode area used in the above two cases. Beveling a carbon fiber on a 45° angle creates an elliptical surface with major and minor radii of 3.5 and 2.5 μM, respectively. Area differences between the total electrode area (calculated from the average of the major and minor radii) and the vesicular area suggest that vesicular concentrations should be ~0.061% of their true value when compared to measurements in standard solution.

CONCLUSIONS

The species monitored during amperometric monitoring of exocytosis at PC12 cells has been identified as catecholamine by pharmacological evidence and by comparison of cyclic voltammograms with that of DA in standard solution. While fast-scan rate cyclic voltammetry can be used to identify the secreted substance as catecholamine, it cannot distinguish between catecholamines because their voltammograms are identical for the conditions used in these experiments.[18] However, it has been frequently noted that DA and NE are the only catecholamines present in PC12 cells. Furthermore, the conversion of DA to NE requires the presence of the cofactor ascorbic acid, which is absent from our cellular medium. It has been observed that cells cultured in this manner contain roughly 90% DA and 10% NE.[28] Thus, it appears likely that DA is the catecholamine being monitored during these experiments.

The ability to perform extracellular voltammetry over a single PC12 cell is a significant achievement since PC12 cells release relatively small amounts of transmitter. Previous experiments with amperometry demonstrate that the population of vesicles released from PC12 cells is unimodal[29] with the average being 190 zmol.[24] The relatively infrequent appearance of voltammograms (77 from 29 cells) collected in the current experiment suggests that only larger events are detected by voltammetry. Nevertheless, the results demonstrate the feasibility of characterizing release events of zeptomole quantities with voltammetry.

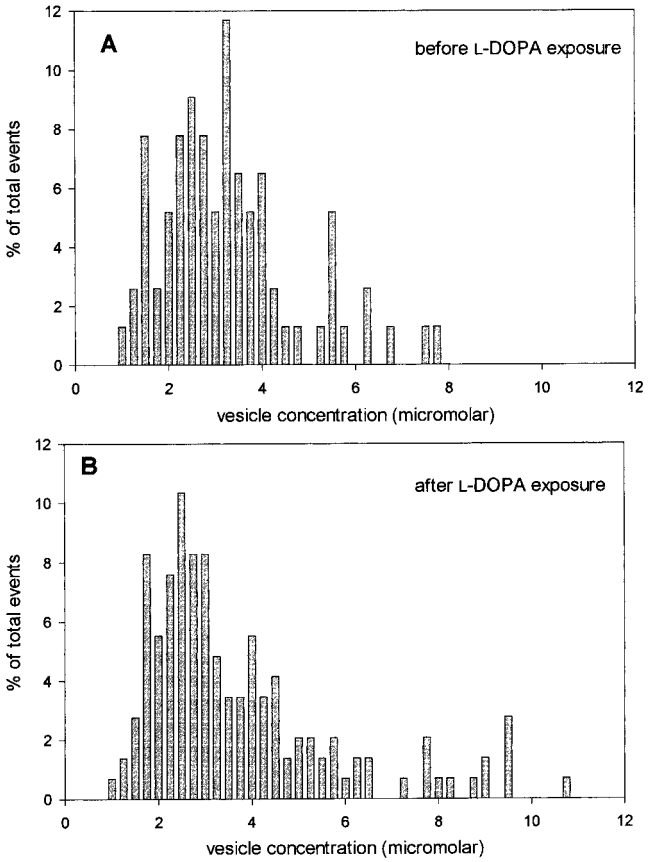

Figure 6. Distributions of vesicular concentrations detected by fast-scan rate cyclic voltammetry following K^+-stimulated exocytosis. (A) Distribution generated from control PC12 cells (n = 29, 77 total events) and (B) distribution generated from L-DOPA-exposed (100 μM for 1 h) PC12 cells (n = 11, 145 total events). For each data set, the peak current of the anodic wave of all events was matched to its respective calibration plot to determine the detected concentration. All data was collected into bins having increments of 0.25 μM and plotted as the percent of the total number of release events detected.

Interestingly, histograms of frequency vs concentration indicate that the intravesicular DA concentration remains relatively unaltered after L-DOPA exposure. This is unlikely to be due only to detecting larger size events using voltammetry since L-DOPA increases the quantal size of the entire unimodal population,[27] which would result in increased filling for the larger events as well. Rather, this result would be consistent with the possibility that vesicles increase in size concomitantly with increased DA synthesis while maintaining the intravesicular concentration or that there are additional factors that serve to limit the rate of transmitter flux through the fusion pore. These conclusions are in agreement with similar suggestions arrived upon by amperometric monitoring of individual events of PC12 cells exposed to L-DOPA.[27] In this previous report, amperometric monitoring revealed current transients with widths at half-height ($t_{1/2}$) that were significantly larger than those of control cells. A larger $t_{1/2}$ is indicative of slower kinetics in the release of vesicular contents, which in turn can be attributed to a larger vesicle size.[20] A comparison between Figures 3B and 4B in this current report adds further support to this argument. The increase in current generally decays more slowly after exposure to L-DOPA, indicating a longer presence of DA under the electrode. Thus, even though

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histograms of frequency vs concentration are similar for both data sets, it appears that the kinetics of release are somewhat slower for L-DOPA-loaded PC12 cells, while a greater amount of catecholamine is released.

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