Published in final edited form as:

Curr Opin Electrochem. 2017 October ; 5(1): 85–91. doi:10.1016/j.coelec.2017.07.005.

Vesicle impact electrochemical cytometry compared to amperometric exocytosis measurements

Johan Dunevall¹, Soodabeh Majdi², Anna Larsson², and Andrew Ewing^{1,2,*}

¹Department of Chemistry and Chemical Engineering, Chalmers University of Technology, 41296 Gothenburg, Sweden

²Department of Chemistry and Molecular Biology, University of Gothenburg, Gothenburg, Sweden

Abstract

Three new tools are discussed for understanding chemical communication between cells and primarily to delve into the content and structure of nanometer transmitter vesicles. These are amperometric measurements of exocytosis, vesicle impact electrochemical cytometry, and intracellular vesicle impact electrochemical cytometry. These are combining in the end nanoscale mass spectrometry imaging to begin determination of vesicle structure. These methods have provided solid evidence for the concept of open and closed exocytosis leading to partial release of the vesicle content during normal exocytosis. They have also been used to discover cases where the fraction of transmitter released is not changed, and other cases where the vesicle transmitter fraction released is altered, as with zinc, thought to alter cognition. Overall, the combination of these methods is showing us details of vesicular processes that would not be measureable without these micro and nano electrochemical methods.

Introduction

Exocytosis is a major cell-to-cell communication process in the nervous system, involving the conversion of an electrical (e.g. action potential) to a chemical signal. Signaling molecules, like neurotransmitters, hormones and/or peptides are stored in cells in organelles called vesicles. During exocytosis, calcium triggers release through SNARE-complex-mediated fusion of vesicles with the cells outer membrane. Consequently, a pore is formed through which the vesicular cargo is released into the extracellular space and is free to interact with a receptor target [1•]. The type of signaling molecule depends on the cell type, for example acetylcholine is released by motor neurons to regulate muscle contraction, insulin is secreted by pancreatic β -cells to regulate the blood glucose level, and norepinephrine, epinephrine and peptides (e.g. chromogranin's and enkephalin) from adrenal cells to regulate heart-rate and blood pressure.

Electrochemical techniques, such as single-cell amperometry (SCA), have proven very important for understanding the release of electroactive molecules like dopamine, norepinephrine, epinephrine, serotonin and histamine from individual vesicles [2–5].

*Corresponding author: Ewing, Andrew (andrew@chalmers.se).

Usually a carbon fiber microelectrode is utilized, which is positioned in close proximity to a cell. Applying a constant positive potential (around 700 mV *versus* Ag/AgCl) allows oxidization of released molecules. Thus, events show up as distinct spikes in a current versus time trace. Integration of the spikes gives the total charge transferred (Q) during the event, which allows for quantification of number of moles released by Faradays law: $N = (Q)/(nF)$, where n is the number of electrons transferred during the redox reaction, and F is Faraday's constant (96,485 C/mol). Due to the high temporal resolution of these experiments (10–200 μ s) detailed information about the dynamics of the release process can be obtained. However, information about the original content of the vesicles cannot be taken from these data.

To fully understand how exocytosis is regulated, additional information about the vesicle must be retrieved. Electrochemistry is a great tool that can easily be miniaturized and therefore also be used in tandem with complimentary techniques. For example, in patch-amperometry a carbon fiber electrode is positioned inside a patch pipette operated in cell-attached mode, capacitive measurements can be conducted in tandem with SCA to get information of number of molecules released and to monitor the change in membrane area [6]. Another example is the use of microfabricated ITO electrodes used in combination with total internal reflection (TIRF) microscopy that can be used to track fluorescently labeled vesicles during the later steps of exocytosis [7–10]. A wide range of labels are available from fluorescent proteins, membrane labels, and small molecules such as false fluorescent neurotransmitters (FFN) [10–14].

In the quest to understand the mechanism of exocytosis, information about the vesicular content must be considered. The Ewing group pioneered this area with a method called flow vesicle electrochemical cytometry (FVEC) [15,16]. The FVEC has later been replaced with two related methods called vesicle impact electrochemical cytometry (VIEC) and intracellular vesicle impact electrochemical cytometry (IVIEC) [17•,18•]. The combination of VIEC and IVIEC with SCA and, to a lesser degree, imaging techniques like NanoSIMS is the topic of this review.

Quantification of vesicle content with electrochemical cytometry

For the purpose of studying vesicular content, electrochemistry and cytometry have been combined in order to measure these fairly small molecular amounts with high enough throughput to be able to make conclusions about the vesicular population. In one such method, FVEC, cellular vesicles are isolated and then separated through electrophoresis in a fused-silica capillary. The end of the capillary is placed in a microfluidic device where the separated vesicles are lysed in close proximity to a carbon fiber microelectrode (Figure 1A). By detecting the oxidative current spikes (Figure 1B), Faraday's law can again be used to calculate the number of molecules contained inside each vesicle [15,16].

A related method, VIEC, has been developed in which the separation step is removed and the electrode placed directly into a suspension of isolated vesicles allowing them to adsorb to the electrode surface. When a potential is applied, the vesicles rupture stochastically and the content of individual vesicles can again be detected via an oxidation current [17•]. An

interesting feature observed in this method is that vesicles rupture without application of a lysis buffer. Evidence suggests that the membrane is disrupted through an electroporation mechanism instead. Despite a small applied potential, if the vesicle membrane is within the double layer at the electrode surface, then the potential field across the membrane will be in the range for electroporation to occur (1.4×10^6 V/cm). The most evident proof of electroporation as the cause of membrane rupture is the potential dependence of the probability of detecting vesicles (Figure 2A and B) [18•]. The number of molecules detected does not seem to change with increased potential, suggesting that electroporation is facilitated but the kinetics of the oxidation reaction is not increased.

For the vesicles to electroporate, they need to be in close proximity of the electrode and the electric field across the electrochemical double layer. As the vesicular membrane is not comprised by pure lipid but also contains large amounts of proteins protruding from the surface, these need to move in order for the membrane to be close enough for electroporation. This is clearly demonstrated when using liposomes as simplified models of vesicles. Liposomes filled with dopamine appear to open as they contact the electrode surface [18•]. They have significantly narrower pore opening and faster oxidation of the loaded transmitters. Liposomes decorated with polyarginine polypeptides have slower pore opening than the non-decorated liposomes, but are still faster than biologically derived vesicles (Figure 2C). This suggests that proteins, or polypeptides, indeed need to diffuse away for the membrane to reach the electrode and for electroporation to occur [18•].

Yet another fascinating aspect observed in VIEC is that excitation of fluorophores inserted in the vesicle membrane increases the number of oxidative spikes [19•]. This can be due to two things: facilitation of adsorption or electroporation of the vesicles. Adsorption could potentially be enhanced through conformational changes in the proteins on the membrane or their localization. These changes could stem from reactive oxygen species (ROS) produced by exciting the fluorophore. Not only might the ROS affect the transmembrane proteins, the membrane itself might also be oxidized and changed through this process. This sort of damage to the membrane would be expected to decrease the bilayer thickness and might include water defects, both of which would make electroporation more likely to occur (Figure 2D). The same effect on the number of spikes observed has also been induced through addition of the known oxidative agents DMSO and H_2O_2 , providing further evidence of how important the state of the membrane is during measurements of this type [19•].

The latest adaptation of this type of measurement is called IVIEC and is based on the same principles as VIEC but is carried out *in situ*. By etching a cylindrical carbon fiber microelectrode, a thin needle shape is obtained with a tip diameter of down to 50–100 nm. This nanotip electrode is then sharp enough to penetrate the cell membrane without causing considerable damage to the cell. Inside the cytosol, vesicles undergo the same process as in VIEC; they adsorb, electroporate, and the content is oxidized, which allows quantification of vesicular content inside the natural environment of the cell. Comparison of IVIEC and VIEC measurements performed on the same cell type (PC12) results in similar values, further suggesting that it is the total vesicular content that is detected [20••], although leakage during vesicle preparation might be an issue. It is important to realize that for these measurements IVIEC is a tool for observing the cellular response of a drug or stimulus on

the vesicular content, whereas VIEC using isolated vesicles bypasses the cell and the regulatory mechanism of individual vesicles can be studied.

Applications of IVIEC versus exocytosis measurements

Li *et al.* combined the IVIEC and SCA techniques to investigate the effect of L-3,4-dihydroxy-phenylalanine (L-DOPA), which is a biochemical precursor to dopamine, on vesicular content versus exocytosis release from PC12 cells [20••]. This was carried out with the same electrode for the two techniques so as to maintain the same signal to noise ratios for similar measurements and comparison (Figure 3) shows that the vesicle content is considerably larger than the amount released. This supports the partial release hypothesis for amperometrically measured exocytosis where some events open fully, but during most normal events the vesicle fuses, opens, and then closes again as is illustrated in for the different modes in Figure 4 [21•,22••]. Cells were also incubated with HEPES physiological saline containing 100 μ M L-DOPA for 2 h and then rinsed three times with HEPES physiological saline prior to the experiments. The total vesicular catecholamine content measured by IVIEC and the amount released were both significantly increased in the L-DOPA-treated cells (Figure 3). Here, the ratio of vesicular content to catecholamine release was not significantly different then that observed for the cells without any treatment (the control cells). These results suggest that the catecholamine content in vesicles does not affect the mechanism of cell exocytosis in terms of the open and closed process.

In another study, the effect of *cis*-diamminedichloroplatinum (cisplatin), an anti-cancer drug, on neurotransmitters released from PC12 cells during exocytosis and vesicular content by SCA and IVIEC, respectively [23••]. PC12 cells were incubated for 3 h with different concentrations of cisplatin. SCA was carried out with a 5 μ m disk carbon fiber electrode, while for IVIEC conical electrodes were utilized. Cisplatin was found to influence the exocytosis ability of a cell in a dose-dependent manner. By treating the cells with low concentration of cisplatin (1–5 μ M), the number of events and the number of released molecules per cell increased while they decreased at higher concentrations (10–100 μ M). To investigate whether these effects were due to changes in vesicular content or not, IVIEC was carried out with the same conditions and vesicular catecholamine content did not change significantly upon cisplatin treatment. Apparently cisplatin influences exocytosis by altering the fusion pore dynamics rather than the vesicle content.

Ren *et al.* studied the effect of micromolar concentrations of zinc on the content of vesicle in PC12 cells versus catecholamine released [24••]. In these series of experiments, PC12 cells were incubated with 100 μ M Zn^{2+} for 3.5 h. In contrast to cisplatin, Zn^{2+} treatment decreases the chemical content of vesicles in the cells as measured by IVIEC; however, the amount of catecholamine released during stimulated exocytosis (by high 130 mM of K^+) remained nearly the same (Figure 5). In addition, zinc changes the rate of neurotransmitter release to lengthen events, which might regulate synaptic strength and plasticity.

The IVIEC and SCA methods have also been used in combination with transmission electron microscopy (TEM) to study the effect of extracellular osmotic pressure on chromaffin cells [25•]. After 10 min incubation in hypertonic solution (730 mOsm/kg) both

the exocytotic activity and amount of release decrease. IVIEC was then carried out on the cells in hypertonic solution and a reduction in vesicular content is observed compared with isotonic solution (310 mOsm/kg). The comparison of these two techniques showed that the relative decline in vesicle content caused by hypertonic solution (59% decrease determined by IVIEC) is consistent with the relative reduction in amount of release during exocytosis (56% decrease determined by exocytosis). The effect of osmotic stress on the amount of catecholamine released during exocytosis appears to be due to the ability of the vesicles to keep the concentration of catecholamine constant under these conditions [26,27].

Lovric *et al.* used a high-resolution mass spectrometry imaging technique called NanoSIMS to image the dopamine content of individual large dense-core vesicles (LDCV) in PC12 cells. They correlated the images with IVIEC and SCA experiments [28••]. In order to image content of the vesicles with NanoSIMS the cells were treated with ¹³C-labeled L-DOPA and/or reserpine. Reserpine is an inhibitor of the vesicular monoamine transporter (VMAT). They treated the cells in three different ways and carried out all three different techniques (NanoSIMS, SCA, and IVIEC) on all groups. First, the cells were incubated with L-DOPA for a short time (LD(S)); second, cells were incubated with reserpine after treatment with L-DOPA (LD + R); and third, cells were incubated with reserpine and then L-DOPA (R + LD). A similar trend in the data was observed among treatment groups between these three different analytical techniques. Results from NanoSIMS showed (Figure 6a) a significant decrease in dopamine content for the LD(S) + R cells as compared to LD(S) cells. IVIEC and exocytosis data showed (Figure 6b and c) that in the LD(S) + R treated cells, the number of dopamine molecules per vesicle did not change compared to control but the difference between the LD(S) + R and LD(S) experiments was comparatively large. Finally, the comparison between the LD(S) and R + LD(S) data showed that preemptively depleting dopamine did not greatly affect the storage capacity of the vesicle. By combining these three methods, they showed that loading and unloading of the vesicular dense core is a kinetically limited process, reflecting both loading of catecholamine and release into and out of the proteinic dense core with a time scale in the order of hours.

Conclusions

The ability to quantify the total neurotransmitter content in vesicles FVEC, VIEC, and IVIEC has provided valuable tools in the quest for understanding chemical communication between cells. This is especially true when combining the methods with SCA of exocytotic events and nanoscale mass spectrometry imaging. These methods have provided solid evidence for the concept of open and closed exocytosis leading to partial release of the vesicle content during normal exocytosis. In some cases, the fraction released is not changed, even when vesicle content is altered. For example, the application of L-DOPA does not change the fraction released nor hyperosmotic solution. In other cases, without or with a change in the vesicle transmitter content the fraction released is altered as with cisplatin and zinc, respectively. These latter two cases also coincide with changes in learning and memory and this might be significant. These concepts should lead to a new area for definition of at least one aspect of synaptic plasticity and short-term memory.

Acknowledgments

The authors thank the many colleagues and collaborators that have contributed to work cited in this review. This work was supported by the [Knut and Alice Wallenberg Foundation](#), the [Swedish Research Council](#), and the [US National Institutes of Health](#). We thank Dr Xianchan Li for the artwork in Figure 4.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

•Paper of special interest

••Paper of outstanding interest.

- 1•• Phan NT, Li X, Ewing AG. Measuring synaptic vesicles using cellular electrochemistry and nanoscale molecular imaging. *Nat Rev Chem*. 2017; 1:s41570–s41017. A review concerning the use of electrochemical, super resolution microscopy, and nanoscale mass spectrometry imaging to determine the chemistry and function of transmitter vesicles.
2. Wightman RM, Jankowski JA, Kennedy RT, Kawagoe KT, Schroeder TJ, Leszczyszyn DJ, Viveros OH. Temporally resolved catecholamine spikes correspond to single vesicle release from individual chromaffin cells. *Proc Natl Acad Sci*. 1991; 88(23):10754–10758. [PubMed: 1961743]
3. Travis ER, Wang YM, Michael DJ, Caron MG, Wightman RM. Differential quantal release of histamine and 5-hydroxytryptamine from mast cells of vesicular monoamine transporter 2 knockout mice. *Proc Natl Acad Sci*. 2000; 97(1):162–167. [PubMed: 10618388]
4. De Toledo GA, Fernández-Chacón R, Fernandez JM. Release of secretory products during transient vesicle fusion. *Nature*. 1993; 363(6429):554–558. [PubMed: 8505984]
5. Chen TK, Luo G, Ewing AG. Amperometric monitoring of stimulated catecholamine release from rat pheochromocytoma (PC12) cells at the zeptomole level. *Anal Chem*. 1994; 66(19):3031–3035. [PubMed: 7978300]
6. Albillos A, Dernick G, Horstmann H, Almers W, De Toledo GA, Lindau M. The exocytotic event in chromaffin cells revealed by patch amperometry. *Nature*. 1997; 389(6650):509–512. [PubMed: 9333242]
7. Amatore C, Arbault S, Chen Y, Crozatier C, Lemaître F, Verchier Y. Coupling of electrochemistry and fluorescence microscopy at indium tin oxide microelectrodes for the analysis of single exocytotic events. *Angew Chem Int Ed*. 2006; 45(24):4000–4003.
8. Meunier A, Jouannot O, Fulcrand R, Fanget I, Bretou M, Karatekin E, Amatore C. Coupling amperometry and total internal reflection fluorescence microscopy at ITO surfaces for monitoring exocytosis of single vesicles. *Angew Chem Int Ed*. 2011; 50(22):5081–5084.
9. Kisler K, Kim BN, Liu X, Berberian K, Fang Q, Mathai CJ, Lindau M. Transparent electrode materials for simultaneous amperometric detection of exocytosis and fluorescence microscopy. *J Biomater Nanobiotechnol*. 2012; 3(2A):243. [PubMed: 22708072]
10. Liu X, Savy A, Maurin S, Grimaud L, Darchen F, Quinton D, Guille-Collignon M. A dual functional electroactive and fluorescent probe for coupled measurements of vesicular exocytosis with high spatial and temporal resolution. *Angew Chem Int Ed*. 2017; 56(9):2366–2370. A modern example of coupling amperometric measurements of exocytosis release with total internal reflection fluorescence (TIRF) to monitor vesicles moving to the cell membrane.
11. Gubernator NG, Zhang H, Staal RG, Mosharov EV, Pereira DB, Yue M, Sulzer D. Fluorescent false neurotransmitters visualize dopamine release from individual presynaptic terminals. *Science*. 2009; 324:1441–1444. [PubMed: 19423778]
12. Lee M, Gubernator NG, Sulzer D, Sames D. Development of pH-responsive fluorescent false neurotransmitters. *J Am Chem Soc*. 2010; 132(26):8828–8830. [PubMed: 20540519]
13. Hettie KS, Liu X, Gillis KD, Glass TE. Selective catecholamine recognition with NeuroSensor 521: a fluorescent sensor for the visualization of norepinephrine in fixed and live cells. *ACS Chem Neurosci*. 2013; 4(6):918–923. [PubMed: 23527575]

14. Klockow JL, Hettie KS, Glass TE. ExoSensor 517: a dual-analyte fluorescent chemosensor for visualizing neurotransmitter exocytosis. *ACS Chem Neurosci*. 2013; 4(10):1334–1338. [PubMed: 23926946]
15. Omiatek DM, Dong Y, Heien ML, Ewing AG. Only a fraction of quantal content is released during exocytosis as revealed by electrochemical cytometry of secretory vesicles. *ACS Chem Neurosci*. 2010; 1(3):234–245. [PubMed: 20368748]
16. Omiatek DM, Bressler AJ, Cans AS, Andrews AM, Heien ML, Ewing AG. The real catecholamine content of secretory vesicles in the CNS revealed by electrochemical cytometry. *Sci Rep*. 2013; 3:1447. [PubMed: 23486177]
- 17•. Dunevall J, Fathali H, Najafinobar N, Lovric J, Wigstrom J, Cans AS, Ewing AG. Characterizing the catecholamine content of single mammalian vesicles by collision–adsorption events at an electrode. *J Am Chem Soc*. 2015; 137(13):4344–4346. First example of vesicle impact electrochemical cytometry to examine the content of biological vesicles isolated from tissue or cells. In this case the vesicles were from the bovine adrenal gland and the data again suggested a larger content than typically observed with amperometry of exocytosis. [PubMed: 25811247]
- 18•. Lovri J, Najafinobar N, Dunevall J, Majdi S, Svir I, Oleinick A, Ewing AG. On the mechanism of electrochemical vesicle cytometry: chromaffin cell vesicles and liposomes. *Faraday Discuss*. 2016; 193:65–79. The mechanism of vesicle impact electrochemical cytometry was examined and modeled showing that liposomes with no proteins open on impact and their contents are electrolyzed faster. The pores during the cytometry experiment were compared for adrenal vesicles, liposomes, and peptide-decorated liposomes. [PubMed: 27711871]
- 19•. Najafinobar N, Lovri J, Majdi S, Dunevall J, Cans AS, Ewing A. Excited fluorophores enhance the opening of vesicles at electrode surfaces in vesicle electrochemical cytometry. *Angew Chem*. 2016; 128(48):15305–15309. Excitation of fluorophores in the membrane of vesicles enhances their frequency of opening, but not the opening event itself. This is suggested to be due to oxidation of the membrane lipids or proteins by the excited state species.
- 20•. Li X, Majdi S, Dunevall J, Fathali H, Ewing AG. Quantitative measurement of transmitters in individual vesicles in the cytoplasm of single cells with nanotip electrodes. *Angew Chem Int Ed*. 2015; 54(41):11978–11982. First example of intracellular vesicle impact electrochemical cytometry, using PC12 cells and nanotip electrodes.
- 21•. Ren L, Mellander LJ, Keighron J, Cans AS, Kurczy ME, Svir I, Ewing AG. The evidence for open and closed exocytosis as the primary release mechanism. *Q Rev Biophys*. 2016; 49:e12. A comprehensive review on the evidence for partial release during normal exocytosis and showing this is mostly what is observed when using amperometric detection. [PubMed: 27659043]
- 22•. Li X, Dunevall J, Ewing AG. Quantitative chemical measurements of vesicular transmitters with electrochemical cytometry. *Acc Chem Res*. 2016; 49(10):2347–2354. A review of the development of vesicle impact electrochemical cytometry and its comparison to amperometric measurements of exocytosis to show that vesicles usually open and close again during this process only releasing part of their contents. [PubMed: 27622924]
- 23•. Li X, Dunevall J, Ewing AG. Using single-cell amperometry to reveal how cisplatin treatment modulates the release of catecholamine transmitters during exocytosis. *Angew Chem Int Ed*. 2016; 55(31):9041–9044. First example to show that a cognition-changing drug, in this case a cancer drug that has a side effect called the “chemobrain”, acts to modulate the way transmitters are released during exocytosis and not by affecting vesicle content.
- 24•. Ren L, Pour MD, Majdi S, Li X, Malmberg P, Ewing AG. Zinc regulates chemical-transmitter storage in nanometer vesicles and exocytosis dynamics as measured by amperometry. *Angew Chem*. 2017; 129(18):5052–5057. An examination of the effect of zinc, known to affect cognition, on exocytosis and vesicle content. Here, zinc was shown to reduce the vesicle transmitter content, but to lengthen the time of exocytosis release so the fraction released is higher.
- 25•. Fathali H, Dunevall J, Majdi S, Lovric J, Cans AS. Osmotic stress reduces vesicle size while keeping a constant neurotransmitter concentration. *Biophys J*. 2017; 112(3):159a. Secreting cells were examined with amperometry and intracellular vesicle impact electrochemical cytometry to compare vesicle content with release in hyperosmotic solution, showing that content and release change concurrently.

26. Colliver TL, Pyott SJ, Achalabun M, Ewing AG. VMAT-mediated changes in quantal size and vesicular volume. *J Neurosci*. 2000; 20(14):5276–5282. [PubMed: 10884311]
27. Sombers LA, Hanchar HJ, Colliver TL, Wittenberg N, Cans A, Arbault S, Ewing AG. The effects of vesicular volume on secretion through the fusion pore in exocytotic release from PC12 cells. *J Neurosci*. 2004; 24(2):303–309. [PubMed: 14724228]
- 28••. Lovric J, Dunevall J, Larsson A, Ren L, Andersson S, Meibom A, Ewing AG. Nano secondary ion mass spectrometry imaging of dopamine distribution across nanometer vesicles. *ACS Nano*. 2016 The techniques of amperometry, intracellular vesicle impact electrochemical cytometry, and nanoSIMS imaging with mass spectrometry were combined to examine the relative amounts of dopamine in the vesicle dense core (protein) and the space around it, as well as the transfer of dopamine between these compartments. The nanoSIMS with about 50 nm spatial resolution was used to image inside single vesicles and the electrochemical techniques were used to monitor the amount of dopamine in the vesicles after successive treatments with reserpine and IL-DOPA. A conclusion from this work is that in the closed vesicle, the transfer between halo and dense core is limited and very slow.

Figure 1.

Flow vesicle electrochemical cytometry. A, Schematic of the microfluidic device where electrophoretically separated vesicles are lysed and detected at a microelectrode [15]. B, Representative trace from detection of dopamine from lysed vesicles. Each spike corresponds to oxidative current from one vesicle [15]. C, Normalized histograms of neurotransmitter content in rat brain vesicles after pharmacological treatment (reserpine in red, control in green, and L-DOPA in blue) [2]. This method can not only quantify the content inside vesicles from a rat brain in a very high throughput manner, but also provide information about the effects of pharmacological manipulation, such as with L-DOPA and reserpine, on an individual vesicle level (C) [16].

Figure 2.

The mechanism of vesicle impact electrochemical cytometry. A, Differences in potential does not change the amount of molecules detected significantly [18]. B, The number of events, however, increase with increasing applied potential, indicating that electroporation might be the mechanism of membrane opening [18]. C, The presence of peptides and proteins seem to determine the dimensions of the pore formed through electroporation [18]. D, Fluorophore excitation and oxidizing species such as DMSO and H₂O₂ potentially damage the membrane and attached proteins causing more adsorption and/or more efficient electroporation [19].

Figure 3. Average number of catecholamine molecules per vesicle as determined by stimulated exocytosis (blank) versus IVIEC (solid) with (19 cells) or without treatment (17 cells) with L-DOPA (100 mM, 2 h) [20]. Error is the standard error of the mean (SEM). **Two-tailed Mann–Whitney rank-sum test, $p < 0.01$.

Figure 4.

Illustration of single cell amperometry and three modes of exocytosis. A, Schematic illustration of exocytosis monitoring using single cell amperometry with a disk carbon fiber microelectrode. Inset, a typical amperometric spike represents an exocytosis event. B, Summary of different modes of exocytosis, including “kiss and run”, “open and closed” or partial release, and complete release with full vesicle distension.

Figure 5.

A, Average peaks obtained from a single cell amperometry. B, IVEC at control (black) and zinc-treated (red) cells. C, Average number of molecules of catecholamine quantified per exocytotic release. D, Vesicle content from control (white) and zinc-treatment (gray) PC12 cells. Pairs of data sets were compared with a Mann–Whitney rank-sum test; ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.1$.

Figure 6.

Partitioning of dopamine between vesicular compartments. A, NanoSIMS measurement of ^{13}C -enrichment, normalized to average vesicle density from each group of samples. B, Number of dopamine molecules detected per vesicle with IVIEC. C, Number of dopamine molecules released per event during exocytosis. Error bars are SEM. One-way ANOVA on ranks: *** $p < 0.001$, n is the number of cells tested. Abbreviations: LD(s), 1.5 h L-DOPA, short exposure; R, reserpine, 1 h exposure.