Using Single-Cell Amperometry and Intracellular Vesicle Impact Electrochemical Cytometry to Shed Light on the Biphasic Effects of Lidocaine on Exocytosis

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ABSTRACT: Single cell amperometry and intracellular vesicle impact electrochemical cytometry were used to examine whether lidocaine can regulate neurotransmitter release or storage for PC12 cells to explain the biphasic effects whereby it can protect neurons and improve cognitive outcome at low concentration, but can cause neurotoxicity at high concentration. We show that lidocaine affects the behavior of PC12 cell exocytosis in a concentration dependent way, which exactly corresponds to its biphasic effects. At a relatively high concentration, it shows a much narrower pore size and a longer-duration fusion pore with less monoamine released than control cells. However, at a relatively low concentration, the fusion pore is open even longer than at high concentration, and with more monoamine released than control cells. Furthermore, intracellular vesicle impact electrochemical cytometry was used to confirm that lidocaine did not change the catecholamines content of the vesicles. These data provide a mechanism for the observed biphasic effects of the drug and suggest that lidocaine influences exocytosis through multiple mechanisms.

INTRODUCTION

Local anesthetics are used to a large extent in clinical surgical practices.[1] Although, the discovery of anesthetics revolutionized surgical procedures, all currently used local anesthetics are, to some extent, neurotoxic, which remains an obvious barrier to patient safety during or after surgical procedures[2, 3]. However, local anesthetics also appear to act as neuroprotective agents in animal studies[4, 5]. A low-dose local of these anesthetics attenuates cerebral ischemia in rats, and can also improve cognitive outcome after global cerebral ischemia and after cardiac surgery for human patients[6]. The mechanistic aspects underlying the neurobiological actions and side-effects of anesthetics are still poorly understood. Moreover, the downstream intracellular changes and changes in intracellular signaling level following local anesthetics is also poorly understood. Although we know that local anesthetics block sodium channels, other ion channels or intracellular signaling pathways can also be affected[7]. At large concentrations of anesthetic, they affect consciousness and can lead to seizures, whereas at low concentration, they have neuroprotective effects and improve cognitive outcome.[5, 8] Clearly, all the effects of local anesthetics cannot be explained by blocking sodium channels. So, we infer the effects of local anesthetics on excitatory or inhibitory neurotransmission might also contribute to their action.

Single-cell amperometry, a real-time quantitative chemical method, can be used to analyze neurotransmitter release during exocytosis[9, 10], the central process in neuronal and hormonal communication. By placing a microdisk electrode on top of a cell, and triggering release with an elevated K^+ ion solution, transmitters exiting via exocytosis can be detected at the electrode. Each exocytotic events is individually collected, leading to recorded peaks. By analyzing all the peaks, parameters related to the geometry and dynamics of the fusion pore and release process can be obtained. This system is fast enough to temporarily resolve dynamics of the neurotransmitter released from individual exocytotic vesicles[11-13]. Intracellular vesicle impact electrochemical cytometry (IVIEC) has been developed to determine catecholamines in single vesicles inside living cells[14]. Here a nanotip electrode is used to pierce the cell and the vesicles in the cell cytoplasm appear to rupture on the electrode surface allowing dopamine in the vesicle to be oxidized and quantified. The IVIEC and amperometry experiments provide fundamental knowledge about the mechanisms of neurotransmitter release, regulation by exocytotic proteins, and the effects of drugs on quantal size and fusion pore dynamics as well as the fraction of transmitter released [15-17].

The study effect of lidocaine on the exocytotic release of neurotransmitter (e.g. dopamine) and vesicular storage using both amperometric measurement of release and IVIEC, respectively, should lead to insights into the mechanism of action for this anesthetic. Thus, in this work, we selected lidocaine as a representative local anesthetic in clinical use. Single cell amperometry and IVIEC methods were used to study the effect of lidocaine on single cell neurotransmitter release and storage, using Pheochromocytoma (PC12) cells as a model. The effect of a 10-min treatment of PC12 cells with different

concentrations of lidocaine on the dynamics, amount of dopamine released during exocytosis and quantal size of vesicles were investigated. Surprisingly, lidocaine treatment is found to modulate exocytosis in different ways depending on the concentration applied, which might explain why lidocaine has biphasic effects pharmacologically.

RESULTS AND DISCUSSION

Measurement of exocytosis. To measure exocytosis, a carbon fiber microelectrode was placed on top of a PC12 cell as shown in Figure 1A and the parameters of the release amount and dynamics were evaluated as in Figure 1B. The cell

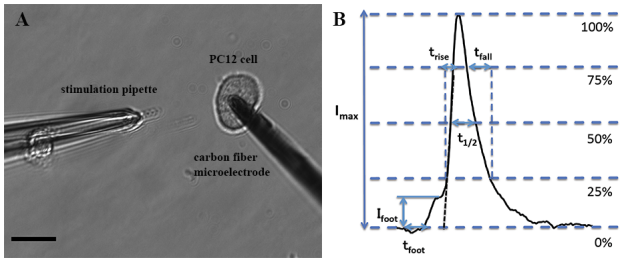

Fig. 1. A) Optical micrograph of the experimental setup for exocytosis. Scale bar: 20 µm. Detection of exocytosis was carried out by applying 700 mV (versus Ag/AgCl reference electrode) to the electrode. B) Scheme to show the different parameters for event analysis .

was stimulated with a 100 mM K^+ solution to initiate exocytosis leading to the recording of amperometric events. Figure 1B shows the parameters obtained from each individual exocytotic spike: t_{rise}, the 25− 75% rise time; $t_{1/2}$, the half peak width; t_{fall}, the 75− 25% fall time; N, the number of catecholamine molecules, from individual events can be quantified with Faraday's equation (N=Q/nF), where n is the number of electrons exchanged in the oxidation reaction (2 for dopamine), Q is the area under the peak and F is Faraday's constant (96485 $Cmol^{-1}$).

PC12 cells were treated for 10 min with isotonic solution containing different concentrations of lidocaine. Typical exocytotic traces obtained from control (10-min incubation in isotonic solution) and lidocaine (0.1 mM and 5 mM lidocaine in isotonic solution for 10 min incubation) are shown in Figure 2A, C and E, respectively. Figure 2 B, D and F are the corresponding averaged peaks obtained from the typical traces for control, 0.1 mM, and 5 mM lidocaine treatment, respectively. In all cases, a train of spikes follows the 5-s stimulation. The exposure of cells to 5 mM lidocaine leads to a wide and short peak whereas exposure to 0.1 mM lidocaine only leads to broad events without changing the height of peak compare with control cells (Figure 2 B, D and F).

Lidocaine affects exocytosis in a dose-dependent manner. Fig. 3 shows the average number of events per cell and the amount of dopamine released increased at lower concentrations of lidocaine (i.e., below 0.1 mM), but displayed a clear decrease (inhibitory effect) at higher concentrations of lidocaine leading to both fewer spikes and molecules released. The specific results obtained from control and cells treated with 0.1 mM and 5 mM lidocaine are summarized in Table 1, which reveal that 0.1 mM lidocaine treatment significantly increases the $t_{1/2}$, t_{rise}, t_{fall}, and the molecules released.

Interestingly, 5 mM lidocaine also leads to a substantial in-

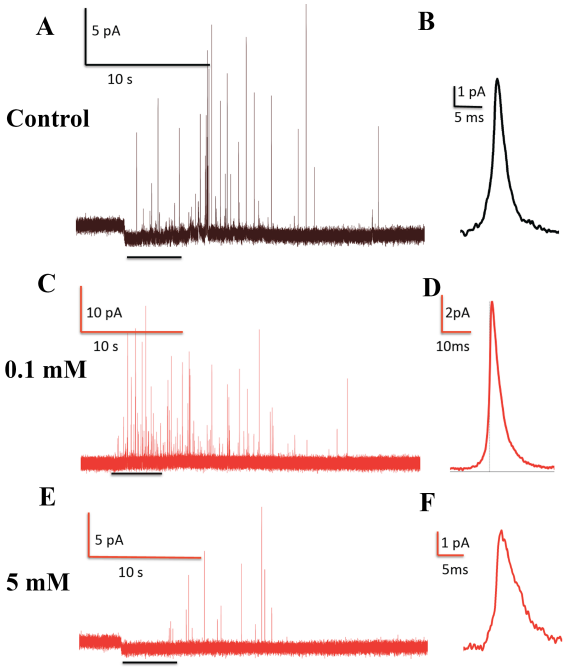

Fig. 2. Typical amperometric traces and corresponding averaged event peaks from a single K^+-stimulated PC12 cell (A, B control; C, D, 0.1 mM lidocaine; E, F 5 mM lidocaine).

crease in $t_{1/2}$ and t_{fall} but a decrease in I_{max} and molecules released without changing the t_{rise}. These results might explain why lidocaine has a biphasic effect on seizure development (proconvulsant and anticonvulsant activity) [18, 19]. A lower concentration of lidocaine has a protective effect on the brain after mild cerebral ischemia[20], but at high concentrations lidocaine is neurotoxic[2]. Mitchell et al. have shown that infusion of an antiarrhythmic dose of lidocaine improves neurologic outcome after cardiac operations, suggesting that at this dose, lidocaine is neuroprotective[21].

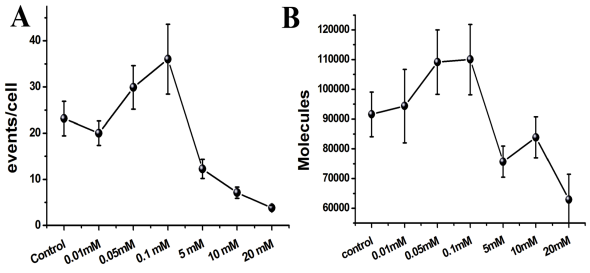

Fig. 3. Exocytosis after lidocaine treatment is dose dependent. A) Mean number of events per cell; B) number of molecules released per vesicle expressed as mean of the median for each cell, in each case after treatment with different concentrations of lidocaine. The numbers of cells for control, 0.01mM, 0.05 mM, 0.1 mM, 5 mM, 10 mM, 20 mM are 41, 22, 24, 20, 43, 17, 15, respectively. The error bar is SEM.

Table 1. Peak analysis parameters obtained from K^+-stimulated PC12 cells (control: 41 cells; lidocaine-treated cells 0.1 mM: 20 cells; 5 mM: 43 cells)[a]

Treatment	$T_{1/2}$/ms	I_{max}/pA	N/10^3 molecules	T_{rise}/ms	T_{fall}/ms
Control	2.4±0.15	7.9 ± 0.5	92 ± 7	0.6 ± 0.003	2.0 ± 0.14
0.1mM Liodocaine	3.0±0.16	7.6 ± 0.5	110 ± 11	0.7 ± 0.003	2.8 ± 0.19
Variations	+25%*	- 0.4%	+20%*	+17%*	+40%**
5mM Lidocaine	2.9±0.2	6.2 ± 0.3	76 ± 5	0.8 ± 0.005	2.2 ± 0.15
Variations	+21%*	-16%*	-17%*	+33%**	+10%

[a] The data are given as the mean of the median ± SEM. Each pair of data is compared with a two-tailed Wilcoxon–Mann–Whitney rank-sum test. **: p<0.01; *: p<0.1.

Vesicle content following lidocaine: IVIEC. IVIEC has been developed in our laboratory to quantify the monoamine content in single mammalian vesicles[14]. To ensure the results observed after the lidocaine treatment are not due to an increase or decrease in vesicular content, this method was used to detect vesicular content for cells with and without lidocaine treatment. We found that vesicular content did not change significantly upon lidocaine treatment (Table 2). Lidaocaine appears to influence exocytosis by altering fusion pore dynamics or other factors rather than the vesicle properties. Additionally, the lower I_{max} is evidence of a narrower fusion pore for release when the cells exposure to 5 mM lidocaine, which results in the amount of released molecules decreasing in comparison with control with even longer release duration time. Assuming an open and closed or partial release mechanism[22, 23], these changes suggest that the cells release a lower fraction of their content after treatment with 5 mM lidocaine, but higher fraction of their content is released for 0.1 mM lidocaine treatment. This again might be important in defining the dose dependence of lidocaine, although it is not known yet what the influence of fraction released from a vesicle is on the strength of an exocytosis signal.

Table 2. Peak analysis parameters obtained from IVIEC in PC12 cells (control: 24 cells; lidocaine treated cells 0.1 mM: 20 cells; 5 mM: 27 cells)[a]

Treatment	$T_{1/2}$/ms	I_{max}/pA	N/10^3 molecules	T_{rise}/ms	T_{fall}/ms
Control	1.9 ± 0.17	24.6 ± 1.3	223 ± 15	0.5 ± 0.06	1.8 ± 0.23
0.1mM Liodocaine	1.5 ± 0.15	36 ± 8.7	207 ± 17	0.45 ± 0.06	1.4 ± 0.18
Variations	-21%	+46%*	-7.2%	-10%	-22.2%
5mM Lidocaine	1.4±0.09	30 ± 1.5	214 ± 16	0.4 ± 0.03	1.3 ± 0.11
Variations	-26%*	+22%**	-4.5%	-20%	-28%*

[a] The data showing here are mean of the median ± SEM. The pairs of data sets compared with a two-tailed Wilcoxon–Mann–Whitney rank-sum test. **: p<0.01; *: p<0.1.

The rate of vesicle closing appears to be affected by lidocaine. To clearly establish the effects of lidocaine on vesicle closing, the decaying part of the current spike was further investigated. Studies from other groups have suggested that the decaying part of the exocytotic spike can be fit either with a single or a double exponential[24]. Further opening and closing of the pore during exocytosis can be inferred using these two exponentials. However, a recent study shows this might also result from the differential diffusion of amines from the dense core versus the halo (or interstitial spaces in the dense core) in the vesicle[25]. Dynamin and actin participated in modulating the dynamics of closing the pore, and are thus involved in control of the decaying part of the exocytotic spike[16, 17]. According to numerical modeling, the diffusion of vesicular contents from the fusion pore might be explained as a single exponential decay. For a perfect single exponential, the pore does not close again. The double exponential decay is consistent with the closing of the fusion pore in the latter stage of the release process. Here, only two situations will be considered: a purely diffusive case (single exponential), where the decay is only controlled by the diffusion of the contents through the fusion pore, and the other case (double exponential) where the release is controlled by the pore closing as well.

When we set the decay of all of the spikes obtained in our experiments to fit with a double decay exponential, then T_1 and T_2 can be obtained which are the characteristic decay times for the dual decay and when these are equal it is a single exponential decay. Plotting T_2 versus T_1, one obtains a normalized distance d for the difference between the second and first order decay. This is equal to $\sin\Theta$, where Θ is the angle between the line (0, 0), (T_1, T_2) and the line (T_1=T_2), as shown in Figure 4A. The value of d is then used to evaluate if the fit is better with a single or double exponential. The equation to calculate d is as follows:

$$d = \sin\Theta = (T_2 - T_1) / [2(T_1^2 + T_2^2)]^{1/2}$$

If d is small, then the peak fit is more suited to a single exponential. Figure 4B shows the results for control and treatment. The y-axis shows the calculated d values, and the x-axis shows the percentage of d values that are below these values. For control experiments, nearly 40% of the peaks show d values below 0.1. However, for 5 mM lidocaine-treated cells, this

point moves to the right. This indicates that 5 mM lidocaine treatment moves the data in the direction of a single exponential decay (or a larger fraction of catecholamine released). For 0.1 mM lidocaine treated cells, about 30% of the peaks show *d* values below 0.1, this point moves to the left compared with control, increasing the widespread of high *d* values, favoring a double exponential decay and a lower fraction of catecholamine released per event. This fact suggests that 0.1 mM lidocaine treatment increases the prevalence of observing a peak generated by a fusion pore closing faster than the diffusion of neurotransmitter from the vesicle. Moreover, the opposite effects on the T_1 and T_2 distributions further suggest that 0.1 mM lidocaine and 5 mM lidocaine affect exocytosis through different pathways and thus have a biphasic effect on seizure development.

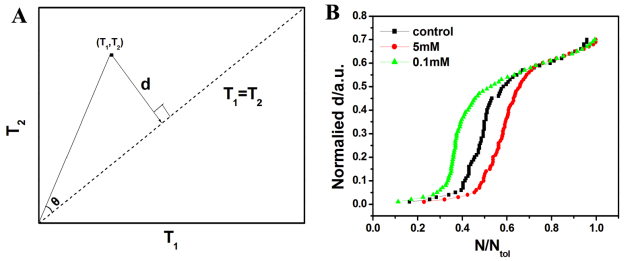

Fig. 4. (A) Scheme showing calculation of the normalized distance *d*. (B) Cumulative plots of the normalized distance *d*, for the control, 0.1 mM and 5 mM lidocaine treatments.

Possible mechanisms of action for lidocaine on exocytosis. We infer the effect of lidocaine on exocytosis is probably via both the protein machinery and changes in the lipid membrane. During Ca^{2+}-regulated exocytosis, numerous proteins have been implicated in this process[26, 27]. Much emphasis has been focused on the importance of the SNARE proteins. Syntaxin1, SNAP-25, and VAMP are the major parts of the core machinery for secretory vesicle docking and fusion[28]. Previous work with PC12 cells has shown that current spikes evoked by Ca^{2+} were markedly reduced in numbers in both BoNT/C1 (light chain of botulinum neurotoxin C1cleaves syntaxin 1) and BoNT/E (neurotoxin E cleaves SNAP-25) transfected cells versus control cells[29]. In our work, lidocaine also exhibited a dose-dependent inhibition of the occurrence of the events release in higher concentrations. As far as we know, there is no direct evidence proves that lidocaine can affect SNARE proteins. However, local anesthetics can inhibit the activity of various ATPases in cell membranes[30] and the observation that the V_0 domain interacts with SNAREs supports a role for V-ATPase in exocytosis[31]. Furthermore, lidocaine can also react with many types of proteins in the cell membrane and cytoplasm. It has been shown to inhibit protein kinase C from rat brain and block platelet gelsolin activation[32], which is a 90-KDa actin filament-capping protein regulating formation of actin filaments[33]. Inhibition of actin polymerization with latrunculin A leads to peaks with longer temporal duration and a greater number of molecules released, which is quite similar to the observation upon 0.1 mM lidocaine treatment[17].

Previous work has shown that anesthetic potency and lipid solubility are correlated. Local anesthetics can affect biological membranes in many ways. Use of tetracaine to disrupt liposomes leads to the formation of mixed micelles of tetracaine and phosphatidylcholine (PC) bilayers in vitro[34, 35]. Tetracaine is another local anesthetics, which has many similarities with lidocaine, e.g. they all have an aromatic ring, intermediate chain, and amine group. Membrane composition is an clearly an important part of the regulation of exocytosis. Previous work in our lab shows that added PC decreases quantal size and slows the dynamics of exocytosis and this result is exactly the same with 5 mM lidocaine treated exocytosis[36]. So, we infer the high dose of 5 mM lidocaine might influence exocytosis via a change in the membrane lipids.

Comparing these results with the data we obtained previously leads us to speculate that the observed effects of lidocaine might be attributed to the influence of lidocaine on vesicle-related proteins at low does and on lipid structure at high dose, Figure 5 summarizes the possible mechanisms for the effects of lidocaine on exocytosis**.** At low lidocaine concentration (left), lidocaine might inhibit actin polymerization resulting in longer release duration with more molecules released in an exocytosis event. At high concentration (right), lidocaine might cause changes in lipid membrane composition or structure and inhibit ATPase activity, resulting in a more narrow fusion pore with less molecules released and also fewer events.

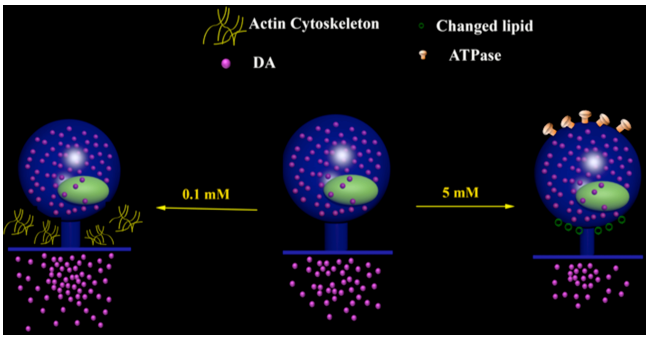

Fig. 5. Proposed scheme for the effect of lidocaine (concentration of drug indicated above the arrows) to exocytosis.

Conclusions. In summary, single cell amperometry and IVIEC have been used to study the effect of lidocaine on exocytosis in PC12 cells and investigate the effects of lidocaine on the catecholamine content of vesicles in PC12 cells, respectively. Although lidocaine does not affect the catecholamine storage, we show that lidocaine has an effect on the release duration and makes it longer apparently by keeping the fusion pore more stable and changing the fraction of transmitter released. However, lidocaine regulates exocytotic release differentially at low and high concentrations, suggesting two different mechanisms of action. We speculate that lidocaine might interact with two proteins, ATPase and actin, shown to be associated with the regulation of partial exocytosis, and change membrane lipid composition, which can affect the dynamics of exocytosis. This work offers a possible explanation of the mechanism of lidocaine at the level of a single cell and provides the means for further understanding and evaluating local anesthetics.

METHODS

Chemicals and Solutions. All chemicals were of analytical grade, and were obtained from Sigma-Aldrich (unless stated otherwise) and used as received. The HEPES physiological saline contained 150 mM NaCl, 5 mM KCl, 1.2 mM MgCl2, 5 mM glucose, 10 mM HEPES, and 2 mM CaCl2. The K^+ stimulation solution consisted of 55 mM NaCl, 100 mM KCl, 1.2 mM MgCl2, 5 mM glucose, 10 mM HEPES, and 2 mM

CaCl2. All solutions were made using 18 MΩ cm water from a Millipore purification system, and the solution pH was adjusted to 7.4 with concentrated (3 M) NaOH.

Fabrication of the Disk Microelectrodes. The disk microelectrode was fabricated as previously described,[37] from 5 μm diameter carbon fibers in borosilicate capillaries (1.2 mm O.D., 0.69 mm I.D., Sutter Instrument Co., Novato, CA, U.S.A.). A micropipette puller (model PE-21, Narishige, Inc., Japan) was used to pull the capillaries. Epoxy (Epoxy Technology, Billerica, MA, U.S.A) was used to seal the electrodes and these were beveled at a 45° angle (EG-400, Narishige Inc., London, UK). Electrodes were tested with cyclic voltammetry (-0.2 to 0.8 V vs. Ag/AgCl, 100 mV/s) in a 100 μM solution of dopamine in PBS (pH 7.4).

Fabrication of Nano-Tip Conical Carbon Fiber Microelectrodes. To fabricate nano-tip conical carbon fiber microelectrodes, a 5 μm carbon fiber was first aspirated into a borosilicate glass capillary which was then pulled into two separate electrodes.[14] The extending fiber was then cut to 100-150 μm with a scalpel under a microscope. Flame etching of the carbon fiber was done by holding the electrodes at the edge of the blue part of a butane flame (Multiflame AB, Hässleholm, Sweden) for about 3 s. When the end of the tip became red, the electrode was immediately pulled out from the flame and checked under the microscope. Electrodes had needle-sharp fiber tips (about 100-200 nm in diameter, 30-100 μm in length) and were sealed with epoxy by touching it to the edge of the fiber at the glass. Electrode were again tested by performing cyclic voltammetry in a solution of 0.10 mM dopamine in PBS (pH 7.4).

Cell Culture. This has been described previously.[14,37] PC12 were from the American Type Culture Collection (Manassas, VA), maintained in phenol RPMI-1640 media (PAA Laboratories, Inc. Australia), supplemented with 10% donor equine serum (PAA Laboratories) and 5% fetal bovine serum Gold (PAA Laboratories) in a 7% CO2, 100% humidity atmosphere at 37 °C. Cells were grown on mouse collagen-coated cell culture flasks (collagen type IV, BD Biosciences, Bedford, MA) and were sub-cultured every 7−9 days. Media was replaced every 2 days throughout the lifetime of all cultures. For single cell exocytotic experiments, PC12 cells were sub-cultured sparsely on mouse collagen coated culture dishes (type IV, BD Biosciences, Bedford, MA) 3−5 days before the experiment and cell media was replaced every other day.

For lidocaine treatment experiments, the cells were treated with lidocaine by rinsing the dishes three times with isotonic solution and then incubating the cells with isotonic solution containing different concentrations of lidocaine for 10 min in a humidified incubator.

Single Cell Experiments. Electrochemical recordings of exocytotic events from single PC12 cells were performed as previously described on an inverted microscope (IX81, Olympus), in a Faraday cage.[14-18] The carbon fiber working electrode was held at +700 mV versus an Ag/AgCl reference electrode using an Axon 200B potentiostat (Molecular Devices, Sunnyvale, CA). The output was filtered at 2.1 kHz using a Bessel filter and digitized at 5 kHz. Cells were rinsed three times with isotonic solution at 37°C before experiments and were maintained under these conditions throughout the experiment. The glass micropipette containing K^+ stimulating solution was placed 60 μm from the cell. Each cell was then stimulated once with a single 5 s K^+ injection (20 psi) through the micropipette coupled to a microinjection system (Picospritzer II, General Valve Corporation, Fairfield, NJ). The electrode potential was 700 mV versus a Ag/AgCl reference electrode. Cells were exposed to lidocaine by rinsing the dishes 3 times with HEPES saline and incubating the cells for 10 min, in the humid incubator, with a solution of lidocaine in HEPES buffer.

Data Processing and Statistics. All the data processing routines were performed with IgorPro 6.21. The amperometric traces were processed using an IgorPro 6.21 routine originating from David Sulzer's group. The filters for the current and differentiated current traces were 2 and 1 kHz, respectively.

Data processing was similar to that in previous single cell experiments in our laboratory.[11] We used a threshold for peak detection on the differentiated trace that was three times the standard deviation of the noise. All traces were carefully inspected after peak detection and false positive were manually rejected. All peaks larger than 2 pA (about four times the noise of the smoothed signal, between 0.5 and 0.7 pA in our experiments, based on 2–4 s baseline acquisitions at the beginning of the trace) were collected.

For comparison, instead of the median of all the spikes from several cells, the mean of the median of all the parameters calculated from single cells was used. In this way, we can minimize the impact of the cell-to-cell variations, as the value is less sensitive to extremes. Pairs of data sets were compared with two-tailed Mann-Whitney rank-sum test;***:$\mathbf{p<}$0.001, **: $\mathbf{p<}$0.01, *: $\mathbf{p<}$0.1.

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All authors have given approval to the final version of the manuscript.

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ABBREVIATIONS

IVIEC intracellular vesicle impact electrochemical cytometry, PC12 pheochromocytoma.

REFERENCES

(1) Stenovec, M., Lasic, E., Bozic, M., Bobnar, S. T., Stout, R. F., Grubisic, V., Parpura, V., and Zorec, R. (2016) Ketamine Inhibits ATP-Evoked Exocytotic Release of Brain-Derived Neurotrophic Factor from Vesicles in Cultured Rat Astrocytes, *Mol Neurobiol 53*, 6882-6896.

(2) Gold, M. S., Reichling, D. B., Hampl, K. F., Drasner, K., and Levine, J. D. (1998) Lidocaine toxicity in primary afferent neurons from the rat, *J Pharmacol Exp Ther 285*, 413-421.

(3) Johnson, M. E. (2004) Neurotoxicity of lidocaine: Implications for spinal anesthesia and neuroprotection, *J Neurosurg Anesth 16*, 80-83.

(4) Lei, B. P., Cottrell, J. E., and Kass, I. S. (2001) Neuroprotective effect of low-dose lidocaine in a rat model of transient focal cerebral ischemia, *Anesthesiology 95*, 445-451.
(5) Lei, B. P., Kass, I. S., and Cottrell, J. E. (2000) Neuroprotective effect of low-dose lidocaine in a rat model of transient focal cerebral ischemia, *Anesthesiology 93*, U188-U188.
(6) Lei, B., Popp, S., Capuano-Waters, C., Cottrell, J. E., and Kass, I. S. (2004) Lidocaine attenuates apoptosis in the ischemic penumbra and reduces infarct size after transient focal cerebral ischemia in rats, *Neuroscience 125*, 691-701.
(7) Lin, T. Y., Chung, C. Y., Lu, C. W., Huang, S. K., Shieh, J. S., and Wang, S. J. (2013) Local anesthetics inhibit glutamate release from rat cerebral cortex synaptosomes, *Synapse 67*, 568-579.
(8) Brosh-Nissimov, T., Ingbir, M., Weintal, I., Fried, M., and Porat, R. (2004) Central nervous system toxicity following topical skin application of lidocaine, *Eur J Clin Pharmacol 60*, 683-684.
(9) Wightman, R. M., Jankowski, J. A., Kennedy, R. T., Kawagoe, K. T., Schroeder, T. J., Leszczyszyn, D. J., Near, J. A., Diliberto, E. J., and Viveros, O. H. (1991) Temporally Resolved Catecholamine Spikes Correspond to Single Vesicle Release from Individual Chromaffin Cells, *P Natl Acad Sci USA 88*, 10754-10758.
(10) Chen, T. K., Luo, G. O., and Ewing, A. G. (1994) Amperometric Monitoring of Stimulated Catecholamine Release from Rat Pheochromocytoma (Pc12) Cells at the Zeptomole Level, *Anal Chem 66*, 3031-3035.
(11) Wang, J., Trouillon, R., Lin, Y. Q., Svensson, M. I., and Ewing, A. G. (2013) Individually Addressable Thin-Film Ultramicroelectrode Array for Spatial Measurements of Single Vesicle Release, *Anal Chem 85*, 5600-5608.
(12) Lin, Y. Q., Trouillon, R., Safina, G., and Ewing, A. G. (2011) Chemical Analysis of Single Cells, *Anal Chem 83*, 4369-4392.
(13) Trouillon, R., Passarelli, M. K., Wang, J., Kurczy, M. E., and Ewing, A. G. (2013) Chemical Analysis of Single Cells, *Anal Chem 85*, 522-542.
(14) Li, X. C., Majdi, S., Dunevall, J., Fathali, H., and Ewing, A. G. (2015) Quantitative Measurement of Transmitters in Individual Vesicles in the Cytoplasm of Single Cells with Nanotip Electrodes, *Angew Chem Int Edit 54*, 11978-11982.
(15) Li, X. C., Dunevall, J., and Ewing, A. G. (2016) Using Single-Cell Amperometry To Reveal How Cisplatin Treatment Modulates the Release of Catecholamine Transmitters during Exocytosis, *Angew Chem Int Edit 55*, 9041-9044.
(16) Trouillon, R., and Ewing, A. G. (2013) Amperometric Measurements at Cells Support a Role for Dynamin in the Dilation of the Fusion Pore during Exocytosis, *Chemphyschem 14*, 2295-2301.
(17) Trouillon, R., and Ewing, A. G. (2014) Actin Controls the Vesicular Fraction of Dopamine Released During Extended Kiss and Run Exocytosis, *Acs Chem Biol 9*, 812-820.
(18) Rey, E., Radvanyibouvet, M. F., Bodiou, C., Richard, M. O., Torricelli, A., Walti, H., and Olive, G. (1990) Intravenous Lidocaine in the Treatment of Convulsions in the Neonatal-Period - Monitoring Plasma-Levels, *Ther Drug Monit 12*, 316-320.
(19) Garcia, P. A., and Alldredge, B. K. (1994) Drug-Induced Seizures, *Neurol Clin 12*, 85-99.
(20) Nagao, S., Murota, T., Momma, F., Kuyama, H., and Nishimoto, A. (1988) The Effect of Intravenous Lidocaine on Experimental Brain Edema and Neural Activities, *J Trauma 28*, 1650-1655.
(21) Mitchell, S. J., Pellett, O., and Gorman, D. F. (1999) Cerebral protection by lidocaine during cardiac operations, *Ann Thorac Surg 67*, 1117-1124.
(22) Li, X. C., Dunevall, J., and Ewing, A. G. (2016) Quantitative Chemical Measurements of Vesicular Transmitters with Electrochemical Cytometry, *Accounts Chem Res 49*, 2347-2354.
(23) Ren, L., Mellander, L. J., Keighron, J., Cans, A. S., Kurczy, M. E., Svir, I., Oleinick, A., Amatore, C., and Ewing, A. G. (2016) The evidence for open and closed exocytosis as the primary release mechanism, *Q Rev Biophys 49*.
(24) Wang, C. T., Grishanin, R., Earles, C. A., Chang, P. Y., Martin, T. F. J., Chapman, E. R., and Jackson, M. B. (2001) Synaptotagmin modulation of fusion pore kinetics in regulated exocytosis of dense-core vesicles, *Science 294*, 1111-1115.
(25) Oleinick, A., Hu, R., Ren, B., Tian, Z.-Q., Svir, I., and Amatore, C. (2016) Theoretical Model of Neurotransmitter Release during In Vivo Vesicular Exocytosis Based on a Grainy Biphasic Nano-Structuration of Chromogranins within Dense Core Matrixes, *J Electrochemi Soc 163*, H3014-H3024.
(26) Sudhof, T. C. (1995) The Synaptic Vesicle Cycle - a Cascade of Protein-Protein Interactions, *Nature 375*, 645-653.
(27) Calakos, N., and Scheller, R. H. (1996) Synaptic vesicle biogenesis, docking, and fusion: A molecular description, *Physiol Rev 76*, 1-29.
(28) Weber, T., Zemelman, B. V., McNew, J. A., Westermann, B., Gmachl, M., Parlati, F., Sollner, T. H., and Rothman, J. E. (1998) SNAREpins: Minimal machinery for membrane fusion, *Cell 92*, 759-772.
(29) Graham, M. E., Fisher, R. J., and Burgoyne, R. D. (2000) Measurement of exocytosis by amperometry in adrenal chromaffin cells: Effects of clostridial neurotoxins and activation of protein kinase C on fusion pore kinetics, *Biochimie 82*, 469-479.
(30) Bond, G. H., and Hudgins, P. M. (1976) Inhibition of Atpase Activity in Human Red-Cell Membranes by Tetracaine, *Biochem Pharmacol 25*, 267-270.
(31) El Far, O., and Seagar, M. (2011) A role for V‐ATPase subunits in synaptic vesicle fusion?, *J neurochemi 117*, 603-612.
(32) Mikawa, K., Maekawa, N., Hoshina, H., Tanaka, O., Shirakawa, J., Goto, R., Obara, H., and Kusunoki, M. (1990) Inhibitory Effect of Barbiturates and Local-Anesthetics on Protein Kinase-C Activation, *J Int Med Res 18*, 153-160.
(33) Kurth, M. C., and Bryan, J. (1984) Platelet Activation Induces the Formation of a Stable Gelsolin-Actin Complex from Monomeric Gelsolin, *J Biol Chemi 259*, 7473-7479.
(34) Fernandez, M. S. (1980) Formation of Micelles and Membrane Action of the Local-Anesthetic Tetracaine Hydrochloride, *Biochim Biophys Acta 597*, 83-91.
(35) Peetla, C., Stine, A., and Labhasetwar, V. (2009) Biophysical Interactions with Model Lipid Membranes: Applications in Drug Discovery and Drug Delivery, *Mol Pharmaceut 6*, 1264-1276.
(36) Uchiyama, Y., Maxson, M. M., Sawada, T., Nakano, A., and Ewing, A. G. (2007) Phospholipid Mediated Plasticity in Exocytosis Observed in PC12 cells, *Brain Res 1151*, 46-54.
(37) Li, X., Mohammadi, A. S. and Ewing, A. G. (2016) Single Cell Amperometry Reveals Curcuminoids Modulate the Release of Neurotransmitters During Exocytosis from PC12 Cells, *J Electroanal Chem*, *781*, 30-35.