Supporting Information

Quantifying Intracellular Single Vesicular Catecholamine Concentration with Open Carbon Nanopipettes to Unveil the Effect of L-DOPA on Vesicular Structure

Keke Hu+, Kim Long Le Vo+, Amir Hatamie, and Andrew G. Ewing*

anie_202113406_sm_miscellaneous_information.pdf
Supporting information

1. Chemicals and solutions
All reagents were purchased from Sigma-Aldrich unless otherwise specified. Ca²⁺-free isotonic solution (mM): 154 NaCl, 4.2 KCl, 0.7 MgCl₂·6H₂O, 11.2 glucose, 10 HEPES, pH 7.4, ~310 mOsm. Cell culture medium: DMEM/Ham’s F12 1:1 supplemented with 10% (v/v) fetal bovine serum, 1% (v/v) penicillin, 1% (v/v) cytosine β-Darabinofuranoside, 0.1% (v/v) 5-fluoro-2'-deoxyuridine.

2. Chromaffin cell isolation
Bovine adrenal glands were obtained from a local slaughterhouse, and the chromaffin cells were isolated as previously described. Briefly, the vein was perfused with Locke’s buffer to clear away blood cells. The medulla was isolated after collagenase (0.2%, Roche, Sweden) treatment, and cells were isolated using a series of homogenization and centrifugation steps. For single cell experiments, ~500000 cells were seeded on collagen (IV) coated dishes (Corning Biocoat, VWR, Sweden) and maintained in a humidified incubator at 37 °C, 5% CO₂ for a maximum of 3 days prior to experiments.

3. L-3,4-dihydroxyphenylalanine (L-DOPA) preparation and cell treatment
A stock solution of L-DOPA was prepared by dissolving L-DOPA in phosphate buffered saline PBS. The solution was kept in the dark and simultaneously purged with argon (6.0, AGA Sweden) because L-DOPA is sensitive to oxygen. Chromaffin cells were exposed to 100 µM L-DOPA by diluting the stock solution in warm cell media. After a 2 h incubation period, cells were rinsed with warm isotonic solution and maintained at 37°C for all amperometric experiments.

4. Fabrication of open carbon nanopipettes (CNPs) with chemical vapor deposition (CVD)
Nanopipettes with tip radii from 50 to 500 nm were pulled from quartz capillaries (1.0 mm o.d., 0.7 mm i.d., Sutter Instrument Co.) with a laser pipette puller (P-2000, Sutter Instruments). Carbon was deposited on the inner wall of quartz nanopipettes with an open channel in the middle by chemical vapor deposition (CVD) using methane as carbon source and argon as the protector, as described previously. In brief, the pulled nanopipettes were placed in a quartz boat in the furnace under an argon flow and the temperature was ramped up to 980°C at a rate of 30°C min⁻¹. At the elevated temperature, a methane/argon mixture (5:3) was passed through the CVD reaction chamber at a net flow rate of 480 sccm (standard cubic centimeters per minute) during heating. The furnace was then cooled under argon. A precise amount of carbon can be deposited by adjusting the duration of the CVD process and controlling tip geometry. Representative SEM images of open CNPs are shown in Figure S1.

5. SEM imaging of open CNPs
SEM images of CNP electrodes were obtained using a scanning electron microscope (SEM) (JEOL model 7000F, resolution of 1.2 nm, accelerating voltage of 2-5 kV, working distance of 10 mm). At first, the electrode was coated with a palladium nano film (30 nm), then the coated CNP electrodes were put inside the SEM sample holder, and then the SEM images were obtained.

6. Intracellular electrochemical measurement
In all amperometric experiments, a potential of +700 mV vs Ag/AgCl was applied using an Axon 200B potentiostat (Molecular Devices, San Jose, CA). For IVIEC measurement, CNP was carefully placed inside the cell by piercing the plasma membrane. Figure S2 shows an image of a 500 nm-radius open CNP inserted into a living chromaffin cell and the corresponding amperometric trace. Current transients were not observed in the initial part of the amperometric trace before CNP insertion, whereas spikes appeared when the CNP was inside the cell. The red arrow indicates the moment the CNP was inserted into the cytoplasm. Usually slight baseline change upon CNPs insertion can be observed and then we are certain of its location. We optically observe the whole process before and after CNP insertion and adjust the position of the CNP if necessary. With the largest-sized (500 nm-radius) open CNPs used in this work, the cells are still living and intact while giving a large number of spikes, as shown in Figure S2.

7. Data acquisition and analysis.
Current transients were recorded and digitized using a Digidata1440A (Molecular Devices) and digitized at 10 kHz or 100 kHz and filtered at 2 kHz using a 4-pole Bessel filter. The data were converted in Matlab (The MathWorks, Inc.) and processed in IgorPro (Wavemetrics, Lake Oswego, OR). Traces were manually checked for potential false detections done by the software. Spike characteristics were determined as number of molecules based on the charge measured in each spike, \( t_{rise} = \) time from 25 to 75% of maximum during the increase of the spike, \( t_{fall} = \) time from 75 to 25% of maximum during the decrease of the spike, \( t_{1/2} = \) full spike width at half-maximum. The medians were calculated from all vesicles at the same size of CNPs.
Figure S1. Representative SEM images of 100 (after intracellular measurement) and 400 nm-radius open CNPs.
**Figure S2.** Image of a 500 nm-radius open CNP inserted into a living chromaffin cell and the corresponding amperometric trace. (The red arrow indicates the moment the CNP was inserted into the cytoplasm; the inset shows the amplification of the spike labelled with the red asterisk).

**Figure S3.** Representative amperometric traces of 200 nm-radius open CNPs obtained from the control cell (upper trace) and L-DOPA-exposed cell (lower trace) at 700 mV vs Ag/AgCl. (The inset shows the amplification of the spikes labelled with the red asterisk).

**Figure S4.** Normalized frequency histograms of intracellular vesicular content of control cells (A) and L-DOPA-exposed chromaffin cells (B) obtained with different-sized open CNPs. (Collected from 7 isolations of chromaffin cells; for the
control cells, 513, 833, 1194 and 1035 spikes obtained with 200, 300, 400 and 500 nm CNPs, respectively. For L-DOPA-exposed cells, 1217, 968, 801 and 1263 spikes obtained with 200, 300, 400 and 500 nm CNPs, respectively.

References

1. O'Connor, D. T., Mahata, S. K., Mahata, M., Jiang, Q., Hook, V. Y., and Taupenot, L. Nat. Protoc. 2007, 5, 1248−1253.
2. Singhal, R.; Bhattacharyya, S.; Orynbayeva, Z.; Vitol, E.; Friedman, G.; Gogotsi, Y. Small Diameter Carbon Nanopipettes. Nanotechnology 2010, 21,015304.
3. Hu, K. K.; Wang, Y. X.; Cai, H. J.; Mirkin, M. V.; Gao, Y.; Friedman, G.; Gogotsi, Y. Anal. Chem. 2014, 86, 8897-8901.
4. Mosharov, E., and Sulzer, D. Nat. Methods 2005, 9, 651–658.