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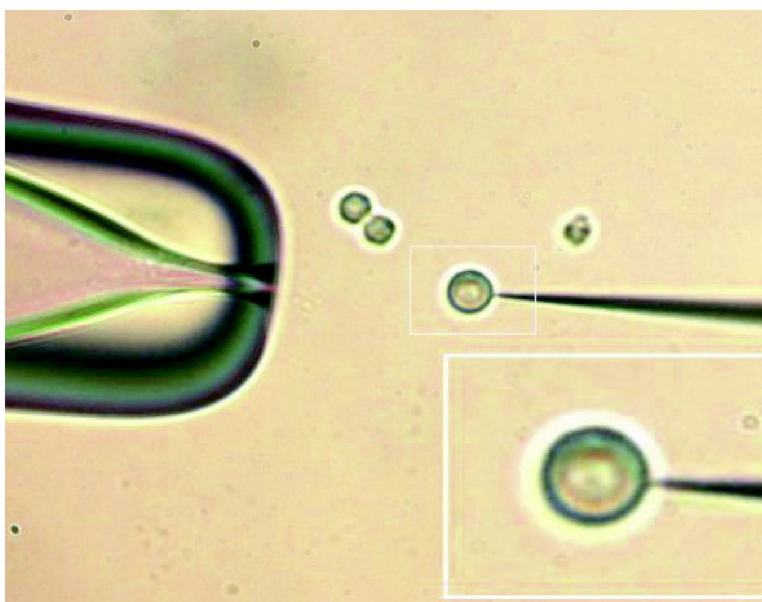

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Monitoring Dopamine Release from Single Living Vesicles with Nanoelectrodes

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As an important biological process, exocytosis and its molecular mechanism have been widely investigated in recent years.[1] The microelectrode electrochemical method has become an ideal tool for the real-time monitoring of exocytosis with the decrease of electrode dimension. The initial electrochemical monitoring of exocytosis was performed by Wightman et al.[2] Since then, many researchers have made efforts to monitor exocytosis from different cells[3] and artificial vesicles.[4] The applied electrochemical probes reported until now are almost all carbon fiber electrodes and modified carbon fiber electrodes with the dimensions at several micrometer levels. Wightman's group[5] developed smaller carbon fiber microelectrodes with a tip diameter of 2 μm, which are capable of monitoring exocytosis on the bovine chromaffin cell's surface of several square micrometers. By combining the pulsed-laser imaging technique, further studies have proven that the neuroendocrine cells have active release zones where release sites are concentrated and most releases occur. The size of the cells is from several micrometers to tens of micrometers, and a cell contains thousands of nanometer-size vesicles[4] in which messengers are stored. The experimental results of conventionally used microelectrodes only reflect the exocytotic characteristics of the whole cell or a part region of the cell. Microelectrodes cannot satisfy the requirements of differentiating the characteristics of each release site and cannot probe into the nanometer-size synaptic cleft for the study of the neurotransmission mechanism. To accomplish such investigations, which are very essential in neuroscience and even in life science, smaller probes with nanometer-size dimensions are required. Several researchers have pointed out this potential trend of research,[6] but so far, no papers have been reported yet.

We previously reported a new and facile method for the fabrication of a low-noise carbon fiber nanoelectrode (tip diameter = 100−300 nm).[7] In this paper, we first used the carbon fiber nanoelctrode (tip diameter = ca. 100 nm) (Figure 1A) as the working electrode and the HEKA EPC-9 patch clamp amplifier as the recording apparatus to amperometrically monitor rat pheochromocytoma (PC12) cells with high spatial resolution.

Figure 1 shows the monitoring of dopamine release from PC12 single cells with a microelectrode and a nanoelectrode. The tip size of a microelectrode is close to the size of a single cell, and the tip almost covered the whole cell (Figure 1B). When the electrode was placed adjacent to the cell, the stimulation by elevated K^+ solution caused exocytosis. The neurotransmitters from most of the covered vesicles diffused to the surface of the electrode to be amperometrically detected, which resulted in a series of current spikes, and each spike corresponds to a vesicle release (Figure 1C). The tip diameter of the nanoelectrode is 100 nm, which is nearly equal to the size of a single PC12 vesicle (the average diameter = 99 nm) (Figure 1D).

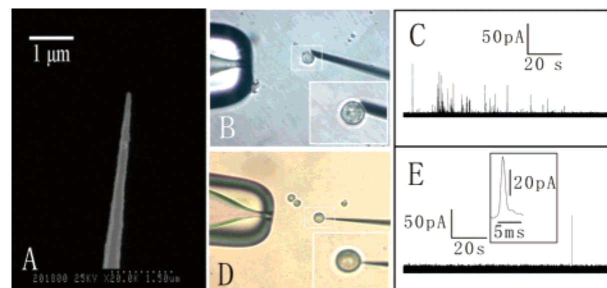

Figure 1. Amperometric monitoring of release from PC12 single cells by a microelectrode (d = 5 μm) and a nanoelectrode (tip diameter = ca. 100 nm). (A) SEM picture of a nanoelectrtde; (B and D) photographs of actual microelectrode−cell and nanoelectrode−cell arrangement, respectively; (C) results from a carbon fiber microelectrode; (E) results from a nanoelectrode (ca. 100 nm). The current spikes are magnified in the inset.

When the nanoelectrode was placed adjacent to the cell (the electrode−cell distance was controlled to less than 0.5 μm, otherwise, the amperometric signal could hardly be detected due to the small area of the electrode), a single current spike was detected with the nanoelectrode, corresponding to the dopamine release from a single vesicle (Figure 1E). It indicates that although many vesicles may release neurotransmitters, only the neurotransmitters from the vesicles just beneath the nanoelectrodes can be detected. The results first demonstrated that the monitoring of dopamine release from single vesicles can be achieved by using a nanoelectrode.

To remove issues regarding cell-to-cell variation and irreproducibility in stimulus delivery, a microelectrode and a nanoelectrode were used simultaneously to monitor the dopamine release from the single vesicles of the same cell. The results (Figure s1, Supporting Information) are similar to that obtained with a nanoelectrode (Figure 1) and further demonstrate that the monitoring of dopamine release from single vesicles with high resolution can only be achieved by the nanoelectrode.

Dopamine release from 121 PC12 cells was monitored a total of 212 times (each lasted 120 s) , and the results are summarized in Table 1. Considering the differences of cell viability, the statistical

Table 1. Monitoring of Dopamine Release from Vesicles Using Nanoelectrodes

current spikes	monitoring time	% of time monitoring	no. of spikes	% of spikes
non-spike	147	69.3	0	0
single spike	31	14.6	31	14.9
multi-spikes	34	16.1	177	85.1
total	212	100	208	100

data were all from the exocytosis that occurred in the cells. It can be seen that 147 out of 212 times monitoring (69.3%) had no current

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spikes, indicating that no vesicles release dopamine beneath the nanoelectrode, and these monitored sites were inactive release sites; 65 of them (30.7%) had current spikes, such sites must be the active release sites. We chose the PC12 cells that released dopamine at least at one site from the nanoelectrode measurements; 87 different sites on the surfaces of 20 cells have been detected, and 30 of 87 different sites had current spikes (34.5%), which was similar to the total statistical result (30.7%). The results demonstrated that most sites on the cell surfaces are inactive, and only a small part of them is active.

The conclusions above were based on the statistics from 121 cells and were consistent with the previously reported results of Wightman's group by using the microelectrode (diameter = 2 μm),[5] which proves the existence and distribution of the active and inactive release zones. The nanoelectrode has also been used to monitor different sites of the same cell. Five randomly selected different sites were detected in Figure 2A; no current spike was obtained at the first three detections, and one current spike was obtained at the last two detections, indicating the existence of the active and inactive release sites on the surface of PC12 cells. The two sites, which were located very close (less than 1 μm), were monitored in Figure 2B, with five current spikes at the first site and three current spikes at the second site, showing that there were different active sites in the same active release zone. These results show that the distribution of vesicle release sites even in the same active zones can be distinguished with high resolution using the nanoelectrode.

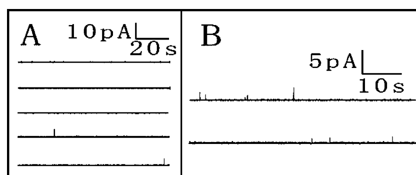

Figure 2. Monitoring the spatial distribution of vesicle release from a PC12 cell with a carbon fiber nanoelectrode (ca. 100 nm). (A) Monitoring of five different sites of a PC12 cell resulted in no current spikes at the first three sites and one at the last two sites, respectively. (B) Monitoring of two different sites of another PC12 cell resulted in five current spikes at the first sites and three at the second, respectively.

In the 65 times of monitoring, 31 had one current spike, and the other 34 had multi-spikes. In each time, the shape and half-width of the current spikes were similar. As shown in Figure 3, four current spikes were obtained when the nanoelectrode was located at the same site of the cell surface and was stimulated by the elevated K^+. Because the nanoelectrode was placed at the same site in the active release zone on the cell surface, the four spikes should correspond to the dopamine release from four vesicles. According to above experimental results, we have demonstrated that multiple vesicles can sequentially release neurotransmitter from the same release site on the surface of cells; that is, after one vesicle fused with the cell membrane and released dopamine, some other vesicles elsewhere were newcomers and then came to the same site to release dopamine, one by one. These experimental results are consistent with the results obtained by total internal reflection microscopy imaging.[8] This is the first report of directly monitoring dopamine release from multiple vesicles at the same release site by the nanoelectrode electrochemical method due to its high spatial resolution.

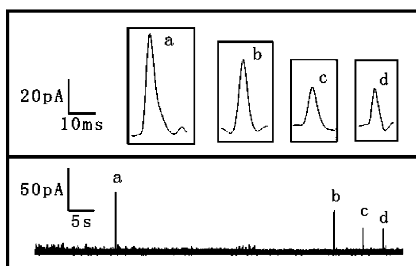

Figure 3. Monitoring of dopamine sequential release from multiple vesicles at the same release site with a nanoelectrode. Four current spikes corresponding to four vesicles sequentially releasing at the same site. Graphs a–d are the magnified pictures of four current spikes.

There were 208 current spikes detected in the 65 active release sites, and there were 31 current spikes (14.9%) occurring in 31 active release sites by single vesicle release and 177 current spikes (85.1%) occurring in the other 34 active sites by dopamine sequential release from multi-vesicles at the same site (24 sites with 2–5 vesicle release, 7 sites with 6–10, and 3 sites with more than 10). The results have demonstrated that the dopamine release from multi-vesicles at the same sites plays the main role in the dopamine release from PC12 cells.

In summary, we first used the nanoelectrodes to monitor of dopamine release from single PC12 cells and have demonstrated that this method can be used to real-time monitor dopamine release with high spatial resolution. The distribution of vesicle release sites with high spatial resolution was monitored, and the distribution of vesicle release sites was differentiated even in the same active release zone. According to the experimental results, we discovered that multiple vesicles can release dopamine at the same site on the surface of cells, and dopamine sequential release from multiple vesicles at the same site played the main role in the dopamine release from PC12 cells. These discoveries provide a new idea and method for deep study of the mechanism of exocytosis and neurotransmission.

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Supporting Information Available: Detailed experimental details and data treatment procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Robert, D. B.; Alan, M. *Physiol. Rev.* **2003**, *83*, 581.

(2) Wightman, R. M.; Jankowski, J. A.; Kennedy, R. T.; Kawagoe, K. T.; Schroeder, T. J.; Leszczyszyn, D. J.; Near, J. A.; Diliberto, E. J.; Viveros, O. H. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 10754.

(3) (a) Chen, G.; Gavin, P. F.; Luo, G.; Ewing, A. G. *J. Neurosci.* **1995**, *15*, 7747. (b) Kennedy, R. T.; Huang, L.; Aspinwall, C. A. *J. Am. Chem. Soc.* **1996**, *118*, 1795. (c) Hochstetler, S. E.; Puopopo, M.; Gustincich, S.; Raviola, E.; Wightman, R. M. *Anal. Chem.* **2000**, *72*, 489. (d) Amatore, C.; Arbault, S.; Bonifas, I.; Bouret, Y.; Erard, M.; Guille, M. *Chemphyschem* **2003**, *4*, 147. (e) Bruns, D.; Jahn, R. *Nature* **1995**, *377*, 62. (f) Elhamdani, A.; Brown, M. E.; Artalejo, C. R.; Palfrey, H. C. *J. Neurosci.* **2000**, *20*, 2495. (g) Staal, R. G. W.; Mosharov, E. V.; Sulzer, D. *Nat. Neurosci.* **2004**, *7*, 341.

(4) (a) Cans, A. S.; Wittenberg, N.; Karlsson, R.; Sombers, L.; Karlsson, M.; Orwar, O.; Ewing, A. G. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 400. (b) Cans, A. S.; Wittenberg, N.; Eves, D.; Karlsson, R.; Karlsson, A.; Orwar, O.; Ewing, A. G. *Anal. Chem.* **2003**, *75*, 4168.

(5) (a) Schroeder, T. J.; Jankowski, J. A.; Senyshyn, J.; Holz, R. W.; Wightman, R. M. *J. Biol. Chem.* **1994**, *269*, 17215. (b) Robinson, I. M.; Finnegan, J. M.; Monck, J. R.; Wightman, R. M.; Fernandez, J. M. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 2474.

(6) (a) Stanford, J. A.; Justice, J. B. *Anal. Chem.* **1996**, *68*, 359A. (b) Adams, R. N. *Anal. Chem.* **1976**, *48*, 1128A. (c) Clark, R. A.; Ewing, A. G. *Mol. Neurobiol.* **1997**, *15*, 1.

(7) Huang, W. H.; Pang, D. W.; Tong, H.; Wang, Z. L.; Cheng, J. K. *Anal. Chem.* **2001**, *73*, 1048.

(8) (a) Zenisek, D.; Steyer, J. A.; Almers, W. *Nature* **2000**, *406*, 849. (b) Steyer, J. A.; Horstmann, H.; Almers, W. *Nature* **1997**, *388*, 474.

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