Primer: spying on exocytosis with amperometry

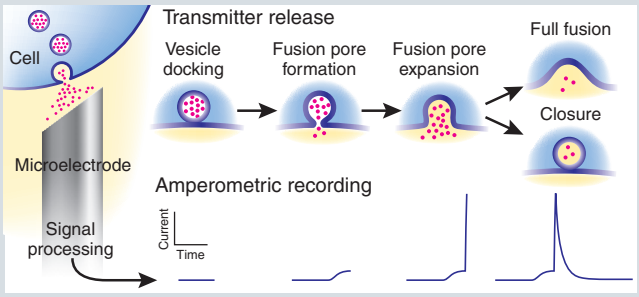
In this issue a review and a protocol describe advances in applying amperometry to biology. Here we provide an overview of amperometry's origins and how it is used to examine the basics of exocytosis.

Regulated exocytosis of biologically active molecules is a central function of many cell types including neuronal and endocrinal secretory cells. Signaling molecules loaded into specialized vesicles in the cytoplasm are released into the extracellular space by regulated vesicle fusion with the plasma membrane. Once released, these neurotransmitters and hormones can bind to receptors on target cells thereby initiating intracellular signaling pathways. This, however, was not known in the 1950s and 1960s, when work by Katz, von Euler and others on signaling across neuronal synapses resulted in the hypothesis that such signaling occurs via the release of quantal amounts of neurotransmitter from small granules in the presynaptic cell. It was not until the development of amperometry over 30 years later that this quantal release of transmitters was directly observed.

Amperometry of biological amines is an adaptation of a technique known for over 80 years in chemistry laboratories as voltammetry or polarography. During voltammetry, a voltage is applied to an electrode immersed in a reaction mixture to oxidize molecules of interest. The use of various profiles of applied voltage during oxidation results in the transfer of electrons to the surface of the electrode, producing a measurable current that varies based on the voltage applied and identity of the oxidized molecule. Analysis of this oxidative current allows one to distinguish between different chemical species that might be present in the reaction mixture.

Following the principal work by the Adams group on the basics of monoamine oxidation at the electrode surface[1,2], Wightman and colleagues reasoned that the combination of constant voltage amperometry and a recently developed small carbon fiber electrode (5-micron diameter)[3] provided all the tools necessary to monitor cellular exocytosis. Positioning the electrode at the surface of a chromaffin cell during stimulation of transmitter release, they were the first to perform presynaptic measurements of exocytotic release of adrenaline and noradrenaline stored within individual dense core vesicles[4]. Since then, amperometry has been extensively used to study exocytosis of various transmitters from different cell types.

With amperometry, the investigator has lost the capacity to distinguish between chemical species, but has gained temporal resolution of less than one millisecond and sensitivity to less than 1,000 molecules. An amperometric experiment produces a current trace in which each spike in the current represents the molecules released from a single vesicle. The area under the spike is used to calculate the amount of released transmitter, whereas its shape contains information about each step of release including creation of the fusion pore, pore expansion and finally, diffusion of the vesicular contents into the extracellular space. Unlike the quantal responses in postsynaptic cells, which typically vary only in frequency, amperometric presynaptic recordings show that individual events also vary in size and



shape. If release were only through a true quantal mechanism, similar to elementary particle quanta from physics, changes in the signal strength coming from the presynaptic cell could be made only by altering the number of release events. The fact that besides the frequency of release, exocytotic events may also vary in other ways suggests that although they are quantal (that is, originating from individual vesicles), each quantum is regulated independently[5,6].

Amperometry is still maturing, and because of the complex nature of the data, methods to analyze amperometric traces can vary considerably from lab to lab, making it difficult to compare data between different sources. In this issue, Mosharov and Sulzer discuss methods for the analysis of amperometric data and present recommendations that are intended to make it easier for researchers to start doing amperometry experiments and to compare data with other labs[7].

Also in this issue, Lindau and colleagues present a protocol for their recently developed patch amperometry method, which combines amperometric detection of monoamines with patch-clamp electrophysiology by placing the carbon-fiber electrode inside a patch pipette[8,9]. This new variation allows one to obtain information on the size of an individual vesicle and the amount and kinetics of transmitter release from the same vesicle.

We hope that the information in these reports helps to open up this exciting field to new researchers and gives new ideas to established specialists.

Daniel Evanko

ACKNOWLEDGMENTS

We thank E. Mosharov for his valuable assistance in the preparation of this primer.

1. Kissinger, P.T., Hart, J.B. & Adams, R.N. *Brain Res.* **55**, 209–213 (1973).
2. McCreery, R.L., Dreiling, R. & Adams, R.N. *Brain Res.* **73**, 15–21 (1974).
3. Gonon, F. *et al. C.R. Acad. Sci. Hebd. Seances. Acad. Sci. D* **286**, 1203–1206 (1978).
4. Leszczyszyn, D.J. *et al. J. Biol. Chem.* **265**, 14736–14737 (1990).
5. Sulzer, D. & Pothos, E.N. *Rev. Neurosci.* **11**, 159–212 (2000).
6. Burgoyne, R.D. & Barclay, J.W. *Trends Neurosci.* **25**, 176–178 (2002).
7. Mosharov, E.V. & Sulzer, D. *Nat. Methods* **2**, 651–658 (2005).
8. Albillos, A. *et al. Nature* **389**, 509–512 (1997).
9. Dernick, G., Gong, L., Tabares, L.W., Alvarez de Toledo, G. & Lindau, M. *Nat. Methods* **2**, 699–708 (2005).