Seeing, Measuring and Understanding Vesicular Exocytosis of Neurotransmitters with “Artificial Synapses”

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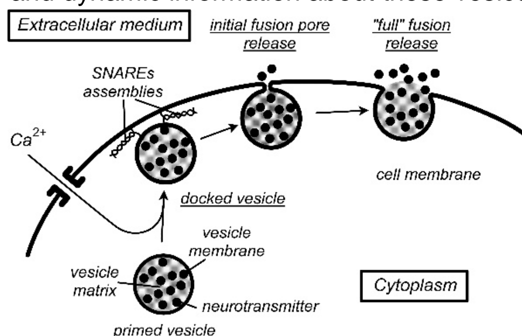
Vesicular exocytosis is a natural nanoscale process. This involves a connection between nanometric vesicles contained inside a cell with the cell membrane occurring by creation of a fusion nanopore across the two membranes through which biologically active molecules contained inside the vesicle are released into the extracellular environment (synaptic cleft, circulating fluids). Despite the minute released amounts (attomoles), single exocytotic events can be studied by means of the ‘artificial synapse’ amperometric method [1], in which a cell is interrogated by a carbon fiber microelectrode collecting and oxidizing released molecules so that the finely-structured electrochemical current tracks quantitatively the exocytotic flux. In the ENS group we investigate essentially chromaffin cells which release adrenaline into the blood stream. Our purpose in this work was to derive topological, energetic and dynamic information about these vesicular exocytotic phenomena.



Such information is obtained by deconvoluting the experimental current by means of simulations involving self-adjustment of time-dependent radius of the fusion nanopore [2-3]. It should be noted, however, that due to the biological variability of the vesicles, the main parameters characterizing a spike (i.e., initial concentration of the neurotransmitter, its diffusion coefficient, vesicle radius, etc.) are not known *a priori*. Nevertheless, reconstruction is possible when at least one of the characteristic dimensions is known as an independent entry. To this end, we resorted to initial fusion nanopore radius values (1.2±0.35 nm), which are well established by patch-clamp measurements [4], for internal calibration of the reconstruction procedure. This resulted in the determination of the average neurotransmitter diffusion rate (D/R_{ves}^2) within the vesicle, which in turn allowed reconstructing the fusion nanopore dynamics from any given spike.

Owing to the large number of spikes in amperometric experiments (several hundred spikes treated) this afforded statistically significant analysis of size distributions of initial fusion pore [4] as well as that in its final stage (full fusion). In turn this provided for the first time experimental access to the potential energy well governing the thermodynamics of such nanosystems suggesting their pure lipidic nature. Conversely, the “full fusion” stage does not end into full fusion but fuse less than ca. 1% of the vesicle membrane surface area with that of the cell since the expansion of the fusion pore appears to be blocked by some mechanical constraint, presumably imposed by the membrane cytoskeleton mesh size. Such quantitative results are in line with previous qualitative observations pointing out that the fusion mechanism is more complex than thought and is most certainly regulated by an active participation of the actin cytoskeleton and possibly also by proteins complexes of the dynamin/dynasaure family. In functional neurons synapses, and using nano-conical carbon fiber electrodes, Kiss-and-Run sequences could also be determined supporting these views [5,6].

Acknowledgements

This work has been supported in part by the CNRS (UMR 8640), Ecole Normale Superieure (ENS, Paris), University Pierre and Marie Curie (UPMC), and the French Ministry of Research. The author thanks Dr. A. Oleinick and Prof. I. Svir for their high involvement in the theory presented, as well as Drs F. Lemaitre, M. Guille Collignon and Prof. Weihua Huang (Wuhan, China) for the experimental material.

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