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Recent advances in Electrochemical Detection of Exocytosis☆

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ABSTRACT

Taking advantages of the analytical properties of ultramicroelectrodes, amperometry at constant potential has been a common technique for investigating exocytosis at the cell level for two decades. The historical experimental configuration, named "artificial synapse", mainly involves a micrometric carbon fiber electrode in the close vicinity of the investigated cell whose exocytotic release thus appears as a succession of amperometric spikes, whose frequency and shapes are particularly informative about the dynamics of the release process, while their areas (charge) directly correspond to the amount of molecules released. While the "single" carbon fiber still contributes to the understanding of the exocytotic mechanism, microsystems and microdevices have blossomed during the recent years and aim to gradually replace the historical experimental configuration by notably allowing coupling with spectroscopies and microscopies (optical, fluorescent). Such changes over the five last years are described and discussed in this review.

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1. Introduction

The basis of intercellular communication in multicellular organisms involves the release of acting molecules into the extracellular medium, i.e. chemical or biochemical messengers from an emitting cell to a target cell. This transmission is mainly achieved by an essential active process, the vesicular exocytosis, notably during the chemical synapse in the neuronal system, through the neurotransmitters release. The importance of this process was recognized by the Nobel Prize of Medicine for 2013 awarded to J. Rothman, R. Schekman and T. Südhof. The molecules are thus packed into secretory vesicles that fuse with the plasma membrane after an appropriate stimulation, thus releasing their content to the outside. The mechanism of exocytosis can be described in several steps. First of all, the cell stimulation induces an intracellular Ca^{2+} increase, thus resulting in the motion of the available secretory vesicles from the cell cytoplasm to the cell membrane at which they dock with the help of SNAREs (soluble N-ethylmaleimide sensitive fusion protein attachment receptors) complexes. Particularly, by overcoming the natural electrostatic repulsions between cell and vesicular membranes, SNAREs thus help the membranes to interact and the subsequent reorganization of phospholipidic bilayers that leads to the formation of a nanometric fusion pore. The release of the vesicular content toward the extracellular medium thus starts and depending on the cell model and the experimental conditions, could lead to the expansion of the pore and a larger flux of the chemical messengers into the extracellular medium.

Three main analytical methods have been developed and used to study exocytosis in real time at the single cell level. Firstly, direct measurements at the single cell level can be achieved through patch-clamp measurements, particularly through the monitoring of the cell membrane surface and conductivity since each vesicle fusion increase the cell membrane surface. [1] Fluorescence microscopy, mainly the observation in evanescent wave field, is based on the use of fluorescent intravesicular markers. [2–4] The imaging of secretory vesicles can therefore be performed in real-time and give access to the status of individual vesicles before and during fusion (displacements, location of the release). Moreover, amperometry at carbon fiber ultramicroelectrode in the artificial synapse configuration also contributes to exocytosis investigation (Fig. 1). In practical terms, a carbon fiber ultramicroelectrode (UME) is positioned at the top of the investigated cell. If a constant and appropriate potential value is applied for oxidizing the molecules released by exocytosis (mainly catecholamines), the exocytotic activity of the cell will be monitored in time and displayed as a succession of amperometric spikes. In this context, each amperometric spike corresponds to an individual exocytotic event. Therefore, the

☆ Dedicated to our great friend and colleague Bernard Tribollet at the occasion of his 65th birthday.

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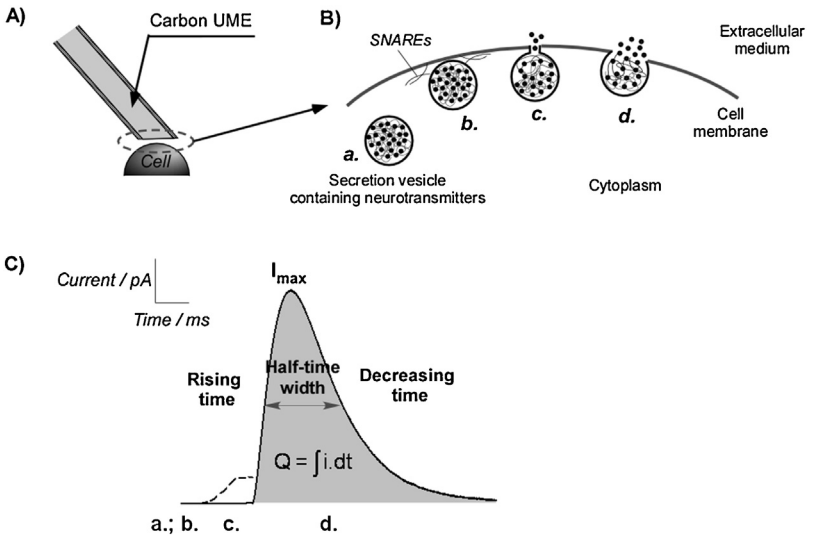

Fig. 1. A) Scheme of the "artificial synapse" configuration. An ultramicroelectrode is positioned in the close vicinity of the emitting cell. If the species released can be oxidized (or reduced) at the electrode surface, amperometry at constant potential allows one to detect a variation of current that features the release in real time. B) Main steps of vesicular exocytosis: after an appropriate stimulation, available vesicles located into the cytoplasm (a.) dock to the cell membrane by the mean of SNAREs assemblies (b.). The subsequent mixing between cell and vesicular membranes induces the formation of a nanometric fusion pore (c.) which can expand and leads to a massive release (d.). C) Typical current spike (correlated to the some steps of exocytosis) recorded by amperometry at a carbon fiber ultramicroelectrode in the "artificial synapse" configuration. The usual extracted parameters (durations, area) are used to decipher the mechanism and its dynamics.

spikes frequency reveals the activity of the cell in the close vicinity of the electrode surface (i.e. at the cell apex if working with a carbon fiber UME) while the detailed analysis of each spike gives access to the dynamics of the secretory events and the amount of neurotransmitters discharged.

Taking benefits from the analytical properties of UMEs (excellent signal-to-noise ratio, low response time), numerous works were devoted to the understanding of the exocytotic process by using the artificial synapse configuration, as reported by some reviews, [5–7] including one by our group. [8] Herein, we reported on the main results obtained with amperometry at ultramicroelectrodes from 1990 to 2008. During the five past years, due to advances in microfabrication procedures, the historical experimental configuration evolved to more sophisticated electrochemical tools. In this review, we thus wish to present the most significant investigations of exocytosis using amperometry, from the works using the artificial synapse configuration with a single carbon fiber electrode to the developments of microsystems and microdevices. The reasons for such new experimental concepts will be also discussed.

2. Electrochemical detection of exocytosis at the single cell level

2.1. Effects of nanoparticles

The artificial synapse configuration, while developed twenty years ago, remains used as a routine technique, particularly for investigations achieved by Christy Haynes and coworkers on the effects on nanoparticles. As a first example, the advantages of UME in artificial synapse configuration using single-cell amperometry were recently shown by studying the effects of various types of nanoparticles uptake on exocytosis of mast cells. [9] First, probing the nanoparticles/cells interactions on mast cells exposed to nanomolar concentrations of serum-coated gold nanoparticles was proposed. The Au nanoparticles accumulate to a small extent within the granular matrix of the vesicles. Effets of this uptake at various nanoparticles concentrations on the dynamic process of serotonin releasing during exocytosis of mast cells were followed by 7 um diameter carbon fiber UME held at +700 mV vs Ag/AgCl reference electrode. However, it has been established that Au nanoparticles presence is not neutral but interferes with the process through altering the number of chemical molecules released as well as the frequency of release. For the 1 nmol.L^{-1} concentration of Au nanoparticles the average spike area significantly increases and the spike half width and frequency decrease. The significant increase of charge released during the fusion pore stage via its observation through the pre-spike feature for the 1 nmol.L^{-1} concentration Au nanoparticles suggests that these particles alter serotonin storage in the granular biopolymer matrix within the vesicles. Second, varying Au nanoparticle exposure time and concentration was assessed by amperometrical recordings performed on a co-culture mast cells/fibroblasts model. [10] Amperometry allows a fine analysis of the secretion on a range of 24–72 h. Secretion frequency (granular release of chemical messengers) increases after 24 h and then decreases after 72 h. The kinetics of serotonin release was impaired with exposure time by slowing with its rise, this being probably due to a change in the matrix swelling kinetics as well as the rate of diffusion within the granular bioplymer. Third, evaluation of the immunotoxicity of mono-2-ethylhexyl phtalate (MEHP) and bisphenol A (BPA) (between 10 and 100 μmol.L^{-1} exposure concentrations) was performed via carbon fiber UME amperometry by the same group on a co-culture model of mouse peritoneal mast cells on a support layer of fibroblasts. [11] A statistically significant decrease in the charge of serotonine released by the mast cells was observed for all the concentrations of exposure for MEHP and BPA. Exocytosis frequency was also altered by reducing for all the BPA conditions but only impaired at the lowest exposure concentration of MEHP. Finally, the role of noble metal nanoparticle ζ-potential effects on immune cell function was also investigated. [12] Carbon fiber amperometry showed a decrease in the total amount of serotonin released during exocytosis for positive charged Au-nanoparticles and negative charged Ag ones. Interestingly the frequency of secretion for these mast cells was affected for both types of charged Au particles when it is the case only for the negatively charged Ag ones, this was attributed to ionic Ag. Though they established that metallic nanoparticles cannot be considered as non-interfering reporters, all these studies evidenced the power and the relevance of

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