Ultramicroelectrode Detection of Adrenaline Releases by Chromaffin Cells: Physical-Chemistry Governs the Dynamics of Exocytosis

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Ultramicroelectrodes have been used for more than a decade to monitor the releases of neurotransmitters by secretory cells (neurons, adrenal cells, pancreatic cells, …).[1] The actual time- and current-resolution of amperometric measurements offers the possibility to characterize the dynamics and the role of the physico-chemical parameters that govern the releases by exocytosis.

Exocytosis of adrenaline by chromaffin cells occurs trough a process involving docking and then fusion of a secretory vesicle to the cytoplasmic membrane of the cell. Fusion proceeds in two main stages. The first one leads to the creation of a stable fusion pore (see figure) between the two membranes and which gives a constant release flux of neurotransmitter (pore release stage). After a few milliseconds, this initial stage proceeds through a sudden enlargement of the pore up to the complete incorporation of the vesicle membrane into that of the cell (full fusion stage) and total exposure of the initial matrix vesicle core to the extracellular medium where the ultramicroelectrode is located. [2]

The precise time-resolved dynamics of the release and of the vesicle membrane during the full fusion could be extracted with a precision never achieved so far by the de-convolution of experimental amperometric currents for each exocytotic secretion event.[2, 3] We have shown that full-fusion events are powered by the swelling of the matrix polyelectrolyte core of the vesicle, although they are kinetically regulated by diffusion in the matrix and dynamics of membranes fusion. A physico-chemical model based on the dynamics of the fusion pore was developed to account for the exocytosis dynamics, it provides a new interpretation of the sudden transition between the pore-release and full fusion stages. This transition occurs when the increasing membrane tension energy due to the constricted internal swelling pressure overcomes the edge energy of the pore, so that the initial fusion pore becomes unstable and is disrupted. This new view predicts that small vesicles (less than 25nm radius containing less than tens of thousand molecules) should always release through a pore while larger vesicles (dense core vesicles) should always end into fusing except if other mechanisms occur to close the pore before fusion. [4]

We designed experiments to test selectively each hypothesis of the model. First, trivalent cations as La^{3+} were used to modify the necessary exchange of adrenaline cations with external cations. Entry of La^{3+} in the vesicle before fusion decreases the rate or stops swelling of the matrix and leads to the abortion or suppression of exocytotic events. Second, variations of the mechanical tension or viscosity of the cell membrane were induced by transient exposure of the cells to hypo- or hyperosmotic media or, by increasing cholesterol membrane content. Our primary results are in very good agreement with the model predictions and provide new insights into the complexity of this important biological phenomena which is exocytosis.

References

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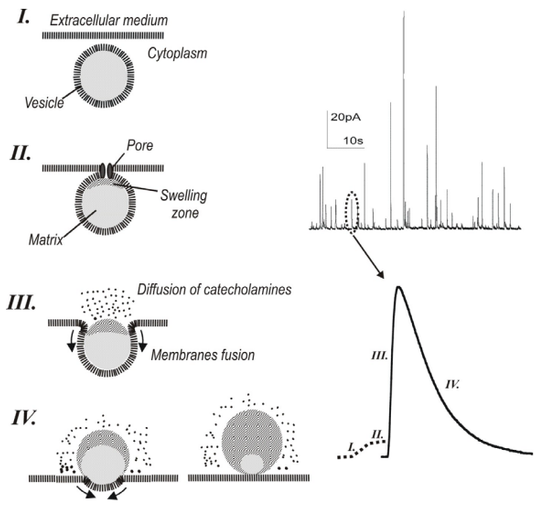

Figure. On the left, are represented the different stages of the exocytosis of a secretory vesicle, as described by our model. On the right, is presented the amperometric monitoring by a carbon fiber ultramicroelectrode of adrenal releases by a chromaffin cell, stimulated by a Ba^{2+} 2mM solution. One event was isolated from the upper trace to display the time-course of the different stages. A foot may be detected for 30% of the peaks and corresponds to the release of molecules through the fusion pore for a few ms before its disruption.