Mathematical Biosciences xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Mathematical Biosciences

journal homepage: www.elsevier.com/locate/mbs

Review

Recent advances in mathematical modeling and statistical analysis of exocytosis in endocrine cells

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ARTICLE INFO

Article history:
Received 19 September 2016
Revised 3 November 2016
Accepted 5 November 2016
Available online xxx

Keywords:
Hormone secretion
Ca^{2+} microdomains
Spatiotemporal Ca^{2+} dynamics
Mixed-effects modeling
Survival analysis
α-cells
β-cells
Pituitary cells
Secretory granules
Large dense-core vesicles

ABSTRACT

Most endocrine cells secrete hormones as a result of Ca^{2+}-regulated exocytosis, i.e., fusion of the membranes of hormone-containing secretory granules with the cell membrane, which allows the hormone molecules to escape to the extracellular space. As in neurons, electrical activity and cell depolarization open voltage-sensitive Ca^{2+} channels, and the resulting Ca^{2+} influx elevate the intracellular Ca^{2+} concentration, which in turn causes exocytosis. Whereas the main molecular components involved in exocytosis are increasingly well understood, quantitative understanding of the dynamical aspects of exocytosis is still lacking. Due to the nontrivial spatiotemporal Ca^{2+} dynamics, which depends on the particular pattern of electrical activity as well as Ca^{2+} channel kinetics, exocytosis is dependent on the spatial arrangement of Ca^{2+} channels and secretory granules. For example, the creation of local Ca^{2+} microdomains, where the Ca^{2+} concentration reaches tens of μM, are believed to be important for triggering exocytosis. Spatiotemporal simulations of buffered Ca^{2+} diffusion have provided important insight into the interplay between electrical activity, Ca^{2+} channel kinetics, and the location of granules and Ca^{2+} channels. By confronting simulations with statistical time-to-event (or survival) regression analysis of single granule exocytosis monitored with TIRF microscopy, a direct connection between location and rate of exocytosis can be obtained at the local, single-granule level. To get insight into whole-cell secretion, simplifications of the full spatiotemporal dynamics have shown to be highly helpful. Here, we provide an overview of recent approaches and results for quantitative analysis of Ca^{2+} regulated exocytosis of hormone-containing granules.

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1. Introduction

The endocrine system is a physiologically important collection of glands that secrete different kind of hormones to control other target cells [1]. Prototype endocrine cells include the pancreatic α- and β-cells, andrenal chromaffin cells, and pituitary cells. Their crucial role in regulating various physiological processes makes the study of the cellular mechanisms in endocrine secretory cells of great importance. In particular, functional impairment of these cells leads to serious diseases such as diabetes [2].

In most endocrine cells, the hormones are contained in secretory granules that, in response to a series of cellular mechanisms culminating with an increase in the intracellular Ca^{2+} concentration, fuse with the cell membrane, a process denoted exocytosis. The main events underlying hormone exocytosis and release are shared with exocytosis of synaptic vesicles underlying neurotransmitter release in neurons [3,4].

The molecular machinery involved in exocytosis is increasingly well understood, and involves isoforms of the SNARE proteins syntaxin and SNAP, which are located in the cell membrane, and VAMP, also called synaptobrevin, inserted into the vesicle/granule membrane [4]. The SNARE proteins can form a so-called SNARE complex, which drives fusion of the two membranes, which – in the case of endocrine cells – allows the hormone molecules contained in the granule to exit the granule and enter the blood stream. SNARE complexes interact with many other proteins, notably Ca^{2+}-sensing proteins such as synaptotagmins, which trigger exocytosis upon Ca^{2+} binding [4]. Thus, the local Ca^{2+} concentration at the Ca^{2+} sensor of the exocytotic machinery is an important determinant of the probability (rate) of exocytosis of the secretory granule. This fact will be a recurrent theme in the present paper.

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http://dx.doi.org/10.1016/j.mbs.2016.11.010
0025-5564/© 2016 Published by Elsevier Inc.

Please cite this article as: M.G. Pedersen et al., Recent advances in mathematical modeling and statistical analysis of exocytosis in endocrine cells, Mathematical Biosciences (2016), http://dx.doi.org/10.1016/j.mbs.2016.11.010

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Depending on their ability to undergo exocytosis, granules in endocrine cells are frequently divided functionally into a readily releasable pool (RRP) and a number of reserve pools [5–9]. The readily releasable granules are immediately available for secretion and typically consists of 1%–5% of the total number of granules in the cell [7]. After exocytosis of these granules, the RRP is resupplied by granules from the reserve pools. However it is still unclear if the refilling process involves physical translocation of granules within the cell, chemical modification of granules already situated at the membrane, recruitment of exocytotic proteins, or a combination of these processes, which are commonly referred to as priming [10–12]. In this paper we will not review mathematical models of the dynamics of the granule pools, but focus only on quantitative approaches to the study in the last step in the life of a secretory granule, i.e., exocytosis (see e.g. [13,14] for recent reviews of models of granule pools in β-cells, and [9] for a discussion of pools and models of synaptic vesicles).

2. Experimental techniques for investigations of exocytosis

2.1. Recording single exocytotic events

Using the patch-clamp technique, individual exocytotic events occurring in a patch of the cell membrane can be observed at discrete steps in membrane capacitance C_m [15–18]. For instance, the fusion of a 300-nm-diameter granule with the plasma membrane yields an electrically detectable step in C_m of 2–3 fF [1,7]. However, the observation of such events is somewhat "lucky punches" in the sense that it is impossible beforehand to estimate the number of granules in the investigated membrane patch, and thus, the experimenter has little idea of the maximal or expected number of exocytotic events that can be observed in response to a stimulus. Hence, rates of exocytosis of the single granules (i.e., the number of events divided by the total number of available granules) cannot be estimated reliably from such data.

Live-cell imaging provides an alternative experimental method for the study of single exocytotic events. For example, two-photon imaging of pancreatic islets bathed in the tracer sulforhodamine-B allows the detection of single fusion events as the tracer enters the granule through the fusion pore, resulting in bright spots below the cell membrane [19–21]. A major advantage of this technique, due to the two-photon microscopy technique is the possibility to monitor exocytosis in cells deep within their natural environment, i.e., within intact pancreatic islets, whereas e.g. capacitance recordings typically are performed on single cells or membrane patches. However, the use of sulforhodamine-B as an extracellular marker does not allow the visualization of secretory granules before they undergo exocytosis. Thus, as for the electrophysiological methods, the single-granule rate of exocytosis cannot be estimated.

In contrast, labeling of the secretory granules with one of several fluorescent markers [22,23] allows the experimenter to follow the single granules with the use of total internal reflection fluorescence (TIRF) microscopy [11,24–31]. TIRF imaging excites fluorescent reporters in a thin (a few hundred nanometer) layer below the cell membrane attached to the coverslip, thus allowing observation of the granules located at the membrane while minimizing the signal from granules deeper within the cells. It is therefore possible to investigate three-dimensional spatial movement of the granules as they approach the membrane, become ready for exocytosis, and eventually undergo exocytosis. Combined with other fluorophores and two-color imaging, it is possible to monitor e.g. Ca^{2+} levels [32,33] or protein abundance [11,29,34] at the individual granules. With such data it is possible to relate rates of exocytosis to signals and molecules controlling single fusion events. An example of such an analysis is given below (Section 4).

2.2. Measuring whole-cell exocytosis as capacitance increases

Monitoring the total cellular amount of exocytosis in response to various stimuli can relatively easy be performed using the patch-clamp technique. As mentioned above, the fusion of granules with the plasma membrane effectively increases the area of the cell membrane. Since the membrane capacitance is proportional to the area, this leads to an increase in whole-cell capacitance, which can be measured using either the whole-cell or the perforated-patch variants of patch-clamping [1,7,35].

Two major stimulation protocols have been applied to investigate rapid exocytosis in various endocrine cells. One option, which is typically used to investigate directly the Ca^{2+} sensitivity of the exocytotic machinery, is to load the cell via the patch pipette with "caged" Ca^{2+}, i.e., Ca^{2+} bound to a light-sensitive buffer, which upon light stimulation is released [1,3]. This so-called "flash release" rapidly increases the Ca^{2+} concentration uniformly in the cell to levels that can be measured simultaneously which Ca^{2+} sensitive probes. By relating the Ca^{2+} levels to the increases in membrane capacitance as a measure of exocytosis, it has been revealed that endocrine exocytosis typically occurs when the Ca^{2+} concentration is raised to tens of μM [36–38]. More recently, pools with higher Ca^{2+} sensitivity ($\sim$ 2 μM) were found in chromaffin [39] and β-cells [40,41]. In chromaffin cells, the capacitance traces can typically be described as a sum of exponentials, which led to a mathematical model describing the exocytotic response as occurring from two different pools that are mutually connected [6]. Recently, this model was simplified by assuming that a priming step was Ca^{2+} dependent, and by confronting the models with experimental data, the authors concluded that the sequential model with release from a single pool effectively described Ca^{2+}-dependent properties of secretion [42].

Although flash-release gives important insight into the Ca^{2+}-sensitivity of the release machinery, the method is unphysiological since Ca^{2+} is raised artificially, and not because of Ca^{2+} influx via voltage-dependent Ca^{2+} channels [3]. A more physiological protocol is to depolarize the cell, which opens Ca^{2+} channels, and consequently triggers Ca^{2+} influx and exocytosis [1,3]. During the depolarization it is possible to measure the Ca^{2+} current, whereas the cell capacitance can only be measured reliably before and after, but not during, the depolarization. To investigate the kinetics of exocytosis it is therefore necessary to apply depolarizing pulses of varying duration. The analysis of this so-called pulse-length protocol was recently performed in details [13] as summarized in Section 6.1.

2.3. Clustered data

Although not uniquely related to the investigations of exocytosis, we wish for a moment to dwell upon the statistical analysis of cell biological experiments. Typically, several observations are performed in the same cell, e.g., subsequent depolarizations to evoke exocytosis, or imaging recordings of many exocytotic events within the same cell. Such experimental data have an inherent clustered structure where observations performed on the same cell correspond to a cluster. It should be expected that measurements from the same individual cell are correlated since they share some specific characteristics of that cell. For example, some cells may be highly-responding, while others are not, and hence, if e.g. a large amount of exocytosis is measured in response to a first depolarization, it should be expected that a subsequent depolarization also triggers much exocytosis. Therefore, it seems reasonable to assume that the recordings from a single cell are independent between each other only if we condition on the cluster, while observations in different cells are independent (independence between clusters).

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