Introduction: Regulated exocytosis

E. Neher*

Max Planck Institute for Biophysical Chemistry, 37077 Goettingen, Germany

ABSTRACT

Calcium ions regulate secretory processes in several ways. Most prominently they (i) trigger the release of vesicle contents rapidly and in a highly cooperative way and they (ii) control priming steps, which prepare vesicles for release. The importance of using assays with high time resolution for separating these distinct roles is pointed out here.

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Ever since the early work on regulated exocytosis by Katz [1] and Douglas [2] calcium ions have been at the center of interest in studies on this process. The topic of this special issue thus certainly is a perfect match for a journal, which carries the name 'Cell Calcium'.

My introduction will handle basically two topics: (i) What have we learnt about the role of intracellular Ca^{++} concentration ($[Ca^{++}]$) in the triggering of exocytosis. This question will mainly deal with the very local $[Ca^{++}]$ signals at the sites of neurotransmitter release, so-called micro- or nanodomains of elevated $[Ca^{++}]$. (ii) The second question relates to other roles of Ca^{++}, mainly in the process of docking and priming, or else in getting vesicles or secretory granules ready for exocytosis. I will emphasize one of the major problems in the functional study of secretory processes, namely how to separate the influence of Ca^{++} on the two steps. My introduction will be biased somewhat towards the two secretory systems, which have been studied in my laboratory over recent years – adrenal chromaffin cells for neuroendocrine secretion and the Calyx of Held as a model synaptic terminal for glutamatergic transmission.

The early work by Del Castillo and Katz [3] already firmly established the role of Ca^{++} in triggering exocytosis. Work on the squid giant synapse employing Ca^{++} chelators then provided first evidence that the Ca^{++} triggering signal is highly localized [4,5] (see [6] for an excellent review on Ca^{++} microdomains). The fact that the dimensions of such microdomains are well below the wavelength of light is probably the reason why for a long time the parameters of these signals, such as amplitude and time course, were not known. The development of caged-Ca^{++} techniques [7], however, allowed one to map out the Ca^{++} sensitivity of the secretory machinery in quite a number of preparations (see Table 1). This revealed high cooperativity (2–5) in most systems and quite a range of apparent affinities for Ca^{++}. The most extensive study of a 'dose–response curve' (release-rate versus $[Ca^{++}]$) was provided by Lou et al. [8], who could show that over a thousand-fold change in $[Ca^{++}]$ glutamate release rates at the Calyx of Held changed by almost seven orders of magnitude. The entire curve was well described by a model, which invokes allosteric interaction between 5 Ca^{++}-binding sites and the release apparatus.

The kinetic characterization of the release process then provided the basis for a new approach towards the long-standing question regarding the time course of the trigger-$[Ca^{++}]$ – or else, which kind of a $[Ca^{++}]$ transient is required for the kinetic model to reproduce the time course of the action potential-induced release. It turned out that sub-millisecond $[Ca^{++}]$ transients of 10–45 μM amplitude have to be postulated, depending on the developmental stage of the synapse and on some additional modeling assumptions [9–11]. Further kinetic modeling of buffered $[Ca^{++}]$ diffusion showed that such transients are typically observed in the vicinity of Ca-channel clusters at distances in the range 20–50 nm [12]. Recent work has shown that this tight coupling most likely is mediated by the active zone protein Rim (see chapter by Han and Schneggenburger, in this issue). Together, recent work on several secretory systems demonstrates that evolution obviously worked both on the sensitivity and speed of the release apparatus as well as on the tightness of its coupling to $[Ca^{++}]$ channels, in order to achieve optimal properties for a given secretory system.

* Correspondence address: Membranbiophysik, Max-Planck-Institut für Biophysikalische Chemie, Am Fassberg 11, 37077 Goettingen, Germany.
Tel.: +49 551 2011630; fax: +49 551 2011688.
E-mail address: eneher@gwdg.de

Table 1
Ca^{2+} dependence of the rate of exocytosis in various secretory cells.

Cell types	Max. rate	$K_{10\%}$	Delay at $K_{10\%}$	Ca cooperativity	Reference
Astrocytes	$1.75\,s^{-1}$	$15\,\mu M$	$250\,ms$	>2	[17]
Pituitary melanotrophs	$25\,s^{-1}$	$20\,\mu M$	ND	3	[18]
Pancreatic β cells	$70\,s^{-1}$	$1\,\mu M$	ND	2	[19,20]
Rod photoreceptors	$400\,s^{-1}$	$20\,\mu M$	ND	4.7	[16]
Chromaffin cells	$1500\,s^{-1}$	$40\,\mu M$	$3\,ms$	3	[21]
Inner hair cells	$1700\,s^{-1}$	$20\,\mu M$	$1.5\,ms$	5	[23]
Bipolar cells	$3000\,s^{-1}$	$80\,\mu M$	$1\,ms$	4	[22]
Inhibitory basket cell	$5000\,s^{-1}$	$20\,\mu M$	$1\,ms$	5	[25]
Calyx of Held	$6000\,s^{-1}$	$30\,\mu M$	$0.3–1\,ms$	5	[24,10]

Source: Most data taken from Neher and Sakaba, 2008 [14]; see [14] for details; approximate data for astrocytes were derived from [17].
Properties of the secretory apparatus of various cell types. Values provided are: maximum release rates at high [Ca^{++}]; $K_{10\%}$, the [Ca^{++}] at which release rate is 10% of its maximum; a measure for the delay in response at this [Ca^{++}] and the Ca^{++} cooperativity, as described from the slope of a double logarithmic plot.

Ca^{++}-triggering, however, is only one of the steps by which secretory system may differ between each other, particularly in their Ca^{++}-dependency. The very early studies by Del Castillo and Katz [3] already distinguished between changes in release probability and changes in the 'number of units available' (Katz's terminology). Early modeling studies, e.g. Elmquist and Quastel [13], made it very clear that availability of releasable vesicles is use-dependent. Later work has established clearly that [Ca^{++}] is a major regulator of vesicle recruitment during sustained secretory activity (see review by Neher and Sakaba [14]). This, indeed, may be a major reason for confusion in the literature, since high time resolution (both with respect to stimulation and the technique to assay release) is required to clearly separate the two processes. It may well be that quite a few Ca^{++}-dependent responses in the past have been attributed to Ca^{++}-triggering, while in reality they reflect Ca^{++}-dependent recruitment of vesicles. This may be the case, whenever the dose–response curves of the two processes overlap. This may readily happen, since Ca^{++}-triggering is typically a highly cooperative process with a sigmoid-relationship (see Fig. 1), while vesicle recruitment is more likely to saturate at higher [Ca^{++}]. Thus, there is a regime at moderate [Ca^{++}] elevation, in which recruitment exceeds release with a concomitant build-up of a readily releasable pool. For higher [Ca^{++}] values, however, release rapidly depletes such a pool with subsequent steady-state release being limited by recruitment. In fact, recent work has shown that both regimes are being utilized physiologically in one and the same cell type: Cone photoreceptors release glutamate in a sustained fashion in darkness. The strength of this release reflects the Ca^{++}-dependent replenishment rate of vesicles [15]. In bright light, however, when cones hyperpolarize, a readily releasable pool of vesicles accumulates, which leads to a burst of release at light offset.

The example of photoreceptor cells clearly points out the importance of tools to separate functional steps in the cycles, which are operative in secretory processes. High time resolution and kinetic analysis are key to such separations, but by itself cannot always provide unambiguous answers. Together with molecular perturbations and pharmacological intervention, however, it should be possible to finally dissect the complex process of vesicle recycling and the fine-tuned regulation of secretion. The articles of this special issue summarize data from a wide spectrum of secretory systems and address many more steps than mentioned here. When reading about the different systems the reader should be able to benefit from this comparative approach, however, he/she should be aware that systems have been optimized for different tasks and seemingly similar phenomena may have different mechanistic roots.

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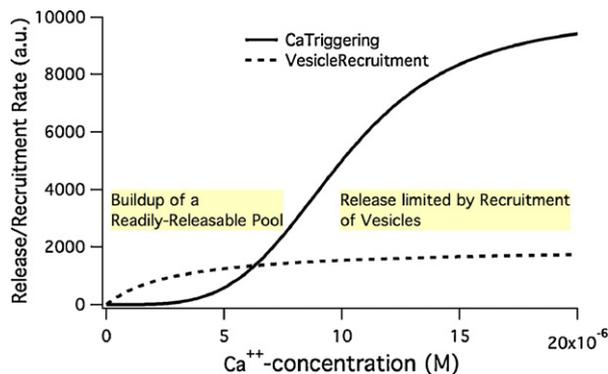
Fig. 1. Ca^{++} triggering and vesicle recruitment have distinct 'dose–response curves'. Schematic plots of reaction rates for Ca^{++}-triggering and vesicle recruitment, both as a function of [Ca^{++}]. The [Ca^{++}] value, at which the two curves cross delineates two regimes: One, for low [Ca^{++}] in which a readily releasable pool can accumulate and another one, in which basically all vesicles, which reach a release-ready state are rapidly consumed. Note that rates are not representative. In particular, recruitment rates are orders of magnitude lower than displayed. Nevertheless, there is a crossing of the 2 curves, as indicated.

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