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Calcium dynamics in the secretory granules of neuroendocrine cells

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ABSTRACT

Cellular Ca^{2+}signaling results from a complex interplay among a variety of Ca^{2+} fluxes going across the plasma membrane and across the membranes of several organelles, together with the buffering effect of large numbers of Ca^{2+}-binding sites distributed along the cell architecture. Endoplasmic and sarcoplasmic reticulum, mitochondria and even nucleus have all been involved in cellular Ca^{2+} signaling, and the mechanisms for Ca^{2+} uptake and release from these organelles are well known. In neuroendocrine cells, the secretory granules also constitute a very important Ca^{2+}-storing organelle, and the possible role of the stored Ca^{2+} as a trigger for secretion has attracted considerable attention. However, this possibility is frequently overlooked, and the main reason for that is that there is still considerable uncertainty on the main questions related with granular Ca^{2+} dynamics, e.g., the free granular $[Ca^{2+}]$, the physical state of the stored Ca^{2+} or the mechanisms for Ca^{2+} accumulation and release from the granules. This review will give a critical overview of the present state of knowledge and the main conflicting points on secretory granule Ca^{2+} homeostasis in neuroendocrine cells.

1. Introduction

Ca^{2+} is an intracellular second messenger able to fulfill a variety of different functions in all cell types with no exception, both excitable and non-excitable cells. Let us just mention muscle contraction, neurotransmitter secretion, fertilization, proliferation, development, learning, memory or cell death [1]. This versatility can only be achieved thanks to a very precise control of its concentration at the subcellular level. In fact, activation of these processes usually takes place as a consequence of transient increases in the cytosolic $[Ca^{2+}]$ in specific subcellular locations, which are variable depending on the process. These hot spots or local $[Ca^{2+}]$ microdomains have a critical importance in Ca^{2+} signaling and their size and dynamics depends on the spatial disposition of the different Ca^{2+} transport systems (channels, exchangers, pumps, etc.) both in the plasma membrane and in intracellular organelles.

Because of the strong Ca^{2+} buffering of the cytosol (free Ca^{2+} constitutes only 1% of the total Ca^{2+} in the cytosol), diffusion of Ca^{2+} through the cytosol is severely limited, particularly during small, physiological, cell stimulations. Intracellular organelles are therefore essential to generate or control local $[Ca^{2+}]$ microdomains at different intracellular locations. The first organelle that was known to have an important role in Ca^{2+} homeostasis was the endoplasmic reticulum (ER) [2]. This organelle behaves as a dynamic Ca^{2+} store. It accumulates Ca^{2+} in its lumen through the SERCA pumps up to near millimolar levels [3], and is able to induce a rapid release of Ca^{2+} to the cytosol via several specific Ca^{2+} channels present in its membrane ($InsP_3$ and ryanodine receptors, mainly). In addition, both the ER and the sarcoplasmic reticulum of muscle cells contain Ca^{2+}-binding proteins with high-capacity and low-affinity that make the total calcium content of the ER to be about 10-fold larger than the free $[Ca^{2+}]_{ER}$. The more abundant, calreticulin in the ER and calsequestrin in the SR, have Ca^{2+}-affinities of 1–4 mM [2], close to the free $[Ca^{2+}]_{ER}$, thus facilitating fast binding and release of Ca^{2+} following the changes in free $[Ca^{2+}]_{ER}$.

Another organelle with an important role in cell Ca^{2+} homeostasis is mitochondria, the organelle responsible of aerobic energy production in the cell. The ability of mitochondria to take up large amounts of Ca^{2+} was known since the sixties. However, the low Ca^{2+}-affinity of the mitochondrial Ca^{2+}uniporter (K_M above 10 μM, 100-fold higher than the resting cytosolic $[Ca^{2+}]$, 100 nM, and 10-fold higher than the usual peak cytosolic $[Ca^{2+}]$ found during cell stimulation, about 1 μM) led to most researchers in the 1970s and 1980s to exclude a significant role in Ca^{2+} homeostasis for this organelle, at least under physiological conditions. Then, in the beginning of the 1990s, recombinant aequorin targeted to the mitochondria showed that mitochondrial $[Ca^{2+}]$ was able to undergo rapid changes during cell activation [4]. That was the origin of a series of works by several research groups demonstrating that mitochondria were very active players in the control of the global cell Ca^{2+} homeostasis. In particular, because of the low-Ca^{2+}-affinity of their Ca^{2+} uptake mechanism, mitochondria are very well

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adapted to take up Ca^{2+} from local high-Ca^{2+} microdomains, such as those formed after the activation of plasma membrane or endoplasmic reticulum Ca^{2+} channels. Mitochondria can therefore modulate important cellular functions such as neurotransmitter secretion by acting as local Ca^{2+} sinks [5].

The subject of this review, the secretory granules, constitutes one of the less known compartments in terms of Ca^{2+} dynamics. It is widely known that the increase in intracellular [Ca^{2+}] levels triggers exocytosis of a pool of docked secretory vesicles in neuronal and neuroendocrine cells. These vesicles are attached to the plasma membrane and are also very close to portions of ER and mitochondria. Thus, it is generally assumed that the Ca^{2+} required for exocytosis may enter from the extracellular medium through several kinds of plasma membrane Ca^{2+} channels [6] or be released from the endoplasmic reticulum through either inositol trisphosphate ($InsP_3$) or ryanodine receptors [7]. Nearby mitochondria may also contribute to modulate the size of the local [Ca^{2+}] microdomain responsible for vesicle fusion and secretion. However, it is hardly mentioned that the main Ca^{2+}-store in some excitable cells resides in the secretory vesicles. In chromaffin cells, secretory granules contain about 60% of the total cellular calcium [8], and the total calcium concentration inside the granules has been estimated to be around 40 mM [9], much higher than that in the ER (about 5–10 mM, see [2]). Of course, an attractive possibility would be that intragranular calcium could contribute to its own secretion by being released through Ca^{2+} channels placed in the granule membrane. In that way, Ca^{2+} would be released precisely in the right place to trigger secretion. What is then the reason for this apparent oversight? As we will discuss in this review, the main reason for that is that there is still little and sometimes contradictory information on the dynamics of Ca^{2+} in the granules. Thus, the main aspects of Ca^{2+} homeostasis in the granules, that is, the free $[Ca^{2+}]_{SG}$ and the mechanisms for Ca^{2+} accumulation and release, are still controversial.

2. The problem of the free and total calcium concentration in the secretory granules

Studies in the 1980s showed that the total calcium concentration in the secretory granules is 40–80 nmol/mg of protein [10,11], which considering a protein/water relationship in the granules of about 0.5 mg/μl [12], implies that the total calcium concentration in the granules is 20–40 mM. Winkler and Westhead [9] calculated a total of 90,000 Ca^{2+} ions for a vesicle with 270 nm internal diameter, which means a concentration of 15 mM. Studies of total calcium made by electron energy loss imaging spectroscopy in PC12 cells show also clearly that dense granules have a high calcium content (>10 mM), with a signal comparable to that obtained in the Golgi complex and some regions of the ER [13]. Accordingly, in PC12 cells, the acidic calcium pool accounted for 170 μmol/l cell water, which corresponds to an internal total concentration of about 30 mM [14]. This high calcium content is not exclusive of chromaffin granules but instead it is also found at similar or even higher values in most types of secretory granules, e.g. zymogen granules of pancreatic acinar cells [15], small synaptic vesicles of the neuromuscular junction [13], insulin granules [16] and many others (see [17] for a review).

Therefore, secretory granules appear to be the organelles with the higher calcium content of the cell. Other organelles with high calcium content are the endoplasmic reticulum and Golgi apparatus. Electron microscopy techniques have shown that the total calcium content of the ER from different cell types is in the range of 5–50 mM, with the highest values in the terminal cisternae of the sarcoplasmic reticulum and mean values of 5–10 mM [2,18], that is, 5–10-fold lower than in the secretory granules. The free [Ca^{2+}] in the ER has been also measured with low-Ca^{2+}-affinity aequorin and is in the 500–800 μM range [7], so that the Ca^{2+}-bound/Ca^{2+}-free relationship in the ER is about 10. In addition, the total calcium distribution in the ER is heterogeneous, with strongly positive cisternae (including the nuclear envelope) lying in the proximity of or even in direct continuity with other, apparently negative cisternae [13]. This heterogeneity must surely reflect the distribution of the Ca^{2+}-binding proteins in the lumen of the ER, as most of the calcium content of the ER is bound with low affinity (K_d = 1–4 mM) to several Ca^{2+}-binding proteins, mainly calreticulin in the ER and calsequestrin in the SR [2,18]. The K_d for Ca^{2+} binding of these proteins is therefore close to the free [Ca^{2+}] in the ER, assuring an efficient binding.

Many studies have tried to estimate the free [Ca^{2+}] in the secretory granules. Bulenda and Gratzl [10] and Haigh et al. [8], using null-point titration techniques, found values of 24 μM and 5.6 μM, respectively, in isolated acidic chromaffin vesicles. In mast cell granules, studies with fluorescent dyes showed a resting intraluminal free [Ca^{2+}] of 25 μM [19]. In the β-cell line MIN6, data obtained with targeted aequorin indicated a free [Ca^{2+}] in the vesicles around 50 μM [20]. These values contrast with the 1.4 μM [Ca^{2+}] value obtained by Mahapatra et al. [21] in PC12 chromaffin granules, using also targeted aequorin. The discrepancy may probably be due to the higher Ca^{2+}-affinity of the aequorin type used in the last study. Because aequorin is consumed faster in high-Ca^{2+} environments, high-Ca^{2+}-affinity aequorin is rapidly consumed in those regions and finally tends to reflect only the behavior of minor compartments with low [Ca^{2+}] [22]. Using an aequorin with lower Ca^{2+}-affinity and correcting for the effect of acidic pH on aequorin luminescence, we have reported free [Ca^{2+}] values of 50–100 μM in chromaffin granules [23] and 20–40 μM in PC12 and INS1 cells [24]. We should note also that the small size of some of the vesicles poses some restrictions to the possible values of free granular [Ca^{2+}]. Let us just mention that for a small synaptic vesicle, with a diameter of 50 nm, 25 μM [Ca^{2+}] would be obtained with only 1 Ca^{2+} ion, and even for a large dense core vesicle of 200 nm diameter, just 2–3 Ca^{2+} ions per vesicle would represent 1 μM [Ca^{2+}] (that would give figures of 100–300 free Ca^{2+} ions per vesicle).

In conclusion, the free [Ca^{2+}] in several types of secretory granules appears to range between 20 and 100 μM. These values are significantly lower, nearly by one order of magnitude, than the free [Ca^{2+}] in the endoplasmic reticulum, which is about 500 μM [3,25]. Given that the total calcium content is larger in the vesicles than in the ER also by nearly one order of magnitude in terms of concentration, this means that the Ca^{2+} bound/Ca^{2+} free relationship is much higher (almost two orders of magnitude) in the vesicles than in the ER, from values of 10–20 in the ER to nearly 1000 in the vesicles. The question then is where all those Ca^{2+}-binding sites are. It is generally assumed that the large calcium storing capacity of the secretory granules is mainly due to the presence of high concentrations (about 2 mM) of the proteins chromogranin A and B and secretogranin [26]. In bovine chromaffin cells, chromogranin A constitutes about 90% of these Ca^{2+}-binding proteins (1.8 mM), and it is able to bind 55 mol of Ca^{2+} per mol of protein, with a K_d of 4 mM [26]. The Ca^{2+}-binding capacity of these proteins is therefore very large and could store up to 100 mM total calcium. However, the affinity is too low if we consider that the free $[Ca^{2+}]_{SG}$ remains below 100 μM. In fact, even considering a free granular [Ca^{2+}] of 100 μM, binding to a protein with a K_d of 4 mM would hardly fill 2.5% of the available binding sites. In our case, this binding would explain 2.5 mM of the stored calcium, that is, less than 10% of the calcium present in the granules.

How can then we explain the large Ca^{2+}-storing capacity of the granules? There are several alternatives: (i) the Ca^{2+} affinity of these Ca^{2+}-binding proteins could be different in the conditions of the secretory granules, that is, at very high protein concentrations (0.5 mg/μl) and in the presence of 500–1000 mM catecholamines