Cell Calcium 51 (2012) 338–341

Contents lists available at SciVerse ScienceDirect

Cell Calcium

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

Vesicular Ca^{2+} mediates granule motion and exocytosis

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ARTICLE INFO

Article history:
Received 29 October 2011
Received in revised form 5 December 2011
Accepted 8 December 2011
Available online 4 January 2012

Keywords:
Amperometry
Bafilomycin
Calcium
Exocytosis
Secretion
TIRFM

ABSTRACT

Secretory vesicles of chromaffin cells are acidic organelles that maintain an increasing pH gradient towards the cytosol (5.5 vs. 7.3) that is mediated by V-ATPase activity. This gradient is primarily responsible for the accumulation of large concentrations of amines and Ca^{2+}, although the mechanisms mediating Ca^{2+} uptake and release from granules, and the physiological relevance of these processes, remain unclear. The presence of a vesicular matrix appears to create a bi-compartmentalised medium in which the major fractions of solutes, including catecholamines, nucleotides and Ca^{2+}, are strongly associated with vesicle proteins, particularly chromogranins. This association appears to be favoured at acidic pH values. It has been demonstrated that disrupting the pH gradient of secretory vesicles reduces their rate of exocytosis and promotes the leakage of vesicular amines and Ca^{2+}, dramatically increasing the movement of secretory vesicles and triggering exocytosis. In this short review, we will discuss the data available that highlights the importance of pH in regulating the association between chromogranins, vesicular amines and Ca^{2+}. We will also address the potential role of vesicular Ca^{2+} in two major processes in secretory cells, vesicle movement and exocytosis.

1. Introduction

Secretory cells are characterized by the presence of secretory storage vesicles that release neurotransmitters and hormones. Adrenal chromaffin cells are commonly used to study the basic mechanisms of neurotransmitter secretion, which involves vesicle sorting, movement and exocytosis. As large concentrations of Ca^{2+} are stored in secretory vesicles, considerable effort has focused on studying the physiological role(s) of vesicular Ca^{2+} in vesicle movement and exocytosis [1–3]. The currently available experimental evidence indicates that the vesicular cocktail constitutes a source of Ca^{2+} that may be involved in regulating vesicle movement and exocytosis. Since the majority of this data is derived from experiments performed in chromaffin cells, we will first describe the most representative organelle of these cells, the chromaffin granule.

1.1. Secretory granules

Secretory granules from chromaffin cells are large, dense, core vesicles (LDCV) similar to those found in many other neuroendocrine cells and sympathetic neurons [4]. Chromaffin granules concentrate transmitters in a highly efficient manner, accumulating catecholamines (500–1000 mM [5,6]) and other soluble components such as ATP (125–300 mM [7]), ascorbate (10–30 mM [8,9]), peptides and chromogranins, thereby forming a condensed protein matrix (~180 mg/mL [10]). Furthermore, secretory granules concentrate H^+ to create an acidic medium that facilitates the accumulation of high concentrations of Ca^{2+}. The mechanisms that mediate the concentration of these elements, despite the large osmotic forces involved, have intrigued researchers for decades.

Chromaffin granules maintain a pH gradient across their membranes of about 2 orders of magnitude (from ≈5.5 on the inside to ≈7.3 in the cytosol). This gradient is maintained by the activity of a specific vesicular H^+-ATPase (V-ATPase; [11,12]), a tightly regulated H^+ transporter present in the membranes of almost all known secretory vesicles [13,14]. This vesicular H^+ gradient acts as an antiporter to accumulate catecholamines through the vesicular monoamine transporter VMAT2 [15], and Ca^{2+} through the H^+/Ca^{2+} antiporter, although most vesicular Ca^{2+} accumulation appears to occur via a SERCA-type Ca^{2+} ATPase [16,17]. The vesicular matrix composed of the distinct proteins and components of the vesicular cocktail appears to play a crucial role in chelation and in reducing the osmotic forces [18], thereby permitting solutes to accumulate at high concentrations [19]. Therefore, most of the intravesicular

Abbreviations: V-ATPase, vesicular H^+-ATPase; VMAT2, vesicular monoamine transporter; LDCV, large dense core vesicles; Cgs, chromogranins; CgA, chromogranin A; CgB, chromogranin B; SgII, secretogranin type II; SERCA, sarcoplasmic reticulum calcium ATPase; CICR, Ca^{2+}-induced Ca^{2+} release.

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doi:10.1016/j.ceca.2011.12.009

solutes are not free but rather, they are associated with other components of the vesicular matrix, the main protein components of which are chromogranins with a pK_a of about 5.5 [18,20].

1.2. Chromogranins are the main protein components of LDCV

Chromogranin A was discovered in the mid sixties [21] as the first of a series of a acidic proteins generically known as granins, of which there are currently 9 examples [22,23]. In chromaffin secretory vesicles, only chromogranins A (CgA) and B (CgB) are really relevant, and to a lesser extent secretogranin II (SgII). The physiological role of Cgs as sources of biologically active peptides has been heavily investigated (see [22]), although granins can also promote granule biogenesis [24,25]. However, in non-secretory cells exogenous granins appear to accumulate in dense compartments that resemble secretory granules [24–27]. Conversely, recent studies using Cg knockout mice have shown that secretory granules persist and secretory responses are maintained in the absence of CgA [19], CgB [28] or both [29]. However, the capacity of secretory granules to accumulate solutes is significantly affected by the absence of Cgs. Although the role of Cgs in the accumulation of other solutes such as Ca^{2+}, H^+ or ATP is still unknown, the ability of Cgs to efficiently bind Ca^{2+} and H^+ suggests that the accumulation of other solutes is also impaired in the absence of Cgs.

As the association of Cgs with other solutes is also pH-dependent [18], it is plausible that intravesicular pH regulates the ability of chromogranin A to form aggregates [30], and that regulating vesicular pH plays an important role in the dynamics of vesicular Ca^{2+} and catechols [16,31,32]. The presence of a functional IP_3 receptor directly coupled to Cgs has been described, which may be involved in the physiological release of Ca^{2+} from vesicles [33]. The association of vesicular membrane-bound IP_3-R to CgA [34] and CgB [35] is strongly dependent on pH.

1.3. Bi-compartmental storage of catecholamines and Ca^{2+}

Intravesicular Ca^{2+} was first implicated in the exocytotic process in 1967 [36], although this hypothesis is yet to be fully accepted by the scientific community. The endoplasmic reticulum has classically been considered as the main internal source of Ca^{2+}, largely because the mobilization of Ca^{2+} from intracellular stores by $InsP_3$ was first described in this organelle. More recent studies have demonstrated the involvement of other cell structures in the uptake, release and cytosolic redistribution of Ca^{2+}, including the mitochondria, nucleus and Golgi apparatus [37–39]. In this respect, secretory vesicles have received modest attention and they are frequently considered little more than a non-functional Ca^{2+} sink. The main argument for this, although with little experimental support, is that vesicular Ca^{2+} is sequestered into the vesicular matrix where it undergoes little turnover. However, recent findings and a reinterpretation of classical data appears to contradict this assumption for several reasons:

i) Secretory granules are the most abundant organelle in chromaffin cells, as well as in many other cell types including pancreatic β-cells, mast cells and lactotrophs. Approximately 30% of the chromaffin cell volume is occupied by about 20,000 granules [40], while β-cells are estimated to contain ≈10,000 insulin granules that occupy 10–20% of their total volume [41].
ii) Chromaffin granules contain far more Ca^{2+} than any other organelle, accounting for about 60% of total Ca^{2+} in chromaffin cells [42,43]. Moreover, calcium is highly concentrated in secretory vesicles. Experiments targeting aequorins to the inside of secretory vesicles have confirmed directly that Ca^{2+} is distributed in two fractions: chelated Ca^{2+} at an estimated concentration of about 40 mM [43]; and the free Ca^{2+} fraction at a concentration of about 50–100 μM [16,43,44]. The free fraction is equilibrated with bound Ca^{2+}, facilitating rapid recovery after acute depletion.
iii) Vesicular Ca^{2+} is the closest source of the cation for granule movement and exocytosis.

Despite these observations, and the crucial role of this cation in processes like vesicle movement and exocytosis, the functional role(s) of vesicular Ca^{2+} has received little attention.

1.4. Mobilization of vesicular Ca^{2+}

Disruption of the pH gradient using protonophores [45] or weak bases [1,2,46] has been used to induce the alkalinization of granules, resulting in the release of Ca^{2+} and catecholamines into the cytosol [46] that can promote acceleration of granule motion and its exocytosis [47]. This effect is shared by clinically used drugs such as the hypotensive agent hydralazine [48], amphetamines [49] and β-adrenergic blockers [50].

Stimuli such as histamine, caffeine and depolarization can mobilize the free Ca^{2+} fraction in vesicles [16,44]. Targeted aequorin studies suggest that intravesicular Ca^{2+} kinetics follow a bi-compartmental model, whereby a large amount of bound Ca^{2+} can rapidly replenish the free Ca^{2+} fraction after depletion induced by SERCA inhibitors (2,5-di-tert-butylhydroquinone (BHQ), cyclopiazonic acid) or pH-disrupting agents [16,44]. In addition, both $InsP_3$- and Ca^{2+}-induced Ca^{2+} release (CICR) occur in chromaffin [16], PC12 [44] and INS-1 secretory vesicles [51]. Ca^{2+}/H^+ antiport activity has also been demonstrated in synaptic vesicles [52–55]. However, the main problem in demonstrating the participation of intravesicular Ca^{2+} in granule movement and exocytosis under physiological conditions is the difficulty in differentiating intravesicular Ca^{2+} from Ca^{2+} from other internal or external sources. All known secretagogues increase free intracellular Ca^{2+} by promoting its entry from extracellular or internal sources. Nevertheless, the vesicular alkalinization observed upon the activation of several second messenger pathways also contributes to the mobilization of vesicular Ca^{2+} and catecholamines (this latter effect having recently been demonstrated using single cell amperometry: [46,47]). Nevertheless, it is plausible that the pH gradient across the vesicular membrane provides the necessary link between physiological stimuli and the regulation of Ca^{2+} and catecholamine release from secretory vesicles.

The presence of these rapid release mechanisms in secretory vesicles is frequently ignored. Conversely, data from experiments using pH-disrupting agents or drugs that act on ryanodine- or IP_3-receptors have been interpreted as reflecting specific events associated to the endoplasmic reticulum or mitochondria, organelles of lesser importance in terms of Ca^{2+} capacity than secretory vesicles, and generally more distant. Thus, in addition to investigating the role of other known organelles, future studies using cell stimulation (via $InsP_3$ receptors, ryanodine receptors or plasma membrane Ca^{2+} channels) should take into account the induction of vesicular Ca^{2+} release. Moreover, other stimuli that activate guanylate cyclase or adenylate cyclase, which alkalinize the vesicular lumen, may also mimic these mechanisms. Given the poor diffusion of Ca^{2+} through the cytosol [56], it is highly plausible that vesicular Ca^{2+} plays an important physiological role in the granule's approach to the membrane [3,57,58] and subsequent exocytosis. To examine this proposal we studied the effects of bafilomycin A1, a potent and highly specific inhibitor of the H^+-ATPase, on vesicle alkalinization and the release of vesicular Ca^{2+} into the cytosol. This Ca^{2+} release increases the lateral movement of chromaffin granules and triggers exocytosis. Although bafilomycin is not a physiological stimulus, these results reveal a novel mechanism for vesicular Ca^{2+} release controlled by the pH gradient.