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Review

Acidic intracellular Ca^{2+} stores and caveolae in Ca^{2+} signaling and diabetes

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ABSTRACT

Acidic Ca^{2+} stores, particularly lysosomes, are newly discovered players in the well-orchestrated arena of Ca^{2+} signaling and we are at the verge of understanding how lysosomes accumulate Ca^{2+} and how they release it in response to different chemical, such as NAADP, and physical signals. Additionally, it is now clear that lysosomes play a key role in autophagy, a process that allows cells to recycle components or to eliminate damaged structures to ensure cellular well-being. Moreover, lysosomes are being unraveled as hubs that coordinate both anabolism via insulin signaling and catabolism via AMPK. These acidic vesicles have close contact with the ER and there is a bidirectional movement of information between these two organelles that exquisitely regulates cell survival. Lysosomes also connect with plasma membrane where caveolae are located as specialized regions involved in Ca^{2+} and insulin signaling. Alterations of all these signaling pathways are at the core of insulin resistance and diabetes.

1. Introduction

Cell metabolism is divided in two processes, anabolism and catabolism, while insulin is a hormone secreted by β cells located in the endocrine part of pancreas; it is also a growth factor and typically promotes anabolism in different type of cells. For instance, accumulation of glycogen in liver and skeletal muscle and triglycerides in adipocytes [1]. On the other hand, autophagy and lysosomes are typically involved in downsizing cells for either recycling of the components or for cell demise in either homeostatic or degenerative processes [2]. It has been proposed that both catabolism and anabolism, at the molecular level, are in close communication by having lysosomes as hubs that organize interactions between AMPK and mTORC1 [3,4]. It is well established that lysosomes are involved in Ca^{2+} regulation and Ca^{2+} signaling in different type of cells and that all these vesicular organelles are in continuous movement both inside cells and toward the plasma membrane, the frontier of cells [5]. Additionally, a lot of information comes from the plasma membrane in different ways; one of these is by endocytosis, being caveolae one of the many forms of endocytosis. Caveolae are specialized plasma membrane structures that are hubs for both cell signaling and Ca^{2+} influx [6].

This review has focused on two main topics, one the role played by acidic compartments in Ca^{2+} regulation and in insulin signaling both in healthy and pathological conditions and two, in the participation of cavoelae in both calcium and insulin signaling in different type of cells, but with particular emphasis in endothelial cells.

2. Acidic intracellular Ca^{2+} stores

It is well established that the main internal Ca^{2+} store is the ER in non-excitable cells and SR in muscle cells; however, there is another intracellular compartment where Ca^{2+} can be stored that is characterized by having an acidic intraluminal pH. The nature of this compartment is mainly vesicular but involves different organelles and it is present from yeast to human cells. In yeast, it has been shown that both the vacuole (a lysosome like compartment) and the ER can accumulate Ca^{2+} [7]. The yeast ER stores Ca^{2+} using a Ca^{2+} ATPase known as PMR, a homolog of the SPCA Ca^{2+} pump present in the Golgi apparatus of animal cells. The vacuole accumulates

Abbreviations: cADPR, cyclic adenosine diphosphate ribose; CICR, calcium-induced calcium release; DAG, diacylglycerol; eNOS, endothelial nitric oxide synthase; ER, endoplasmic reticulum; IP_3R, inositol 1,4,5 trisphosphate receptor; NO, nitric oxide; NAADP, nicotinic acid adenine dinucleotide phosphate; RyR, ryanodine receptor; SOCE, store operated calcium entry; TPC, two pore channel; TRPM, transient receptor potential melastatin channel; TRPML, transient receptor potential mucolipin channel.

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large amounts of Ca^{2+} via two different proteins, one is PMC1, a homolog of PMCA, the plasma membrane Ca^{2+} ATPase of animal cells and the other is a Ca^{2+}/H^+ exchanger (Vcx1). Vacuole contains more than 90% of the Ca^{2+} accumulated by yeast in the form of Ca^{2+} phosphates which implies a very important Ca^{2+} buffering power. Actually, yeast cannot survive in high Ca^{2+} media if both the vacuolar H^+ ATPase and PMC1 have been eliminated [8]. It appears that Ca^{2+} can be released from vacuole via Yvc1, a Ca^{2+} permeable channel homolog of TRPVs ion channels of animal cells [7]. It has also been demonstrated two different intracellular Ca^{2+} stores in S2 cells from *Drosophila melanogaster* using electron probe microanalysis that can be distinguished morphologically and that have large $[Ca^{2+}]$ in the order of mM in resting conditions, i.e. without stimulated Ca^{2+} entry to S2 cells. Importantly, alkalization by incubating cells with NH_4Cl, specifically decreases the Ca^{2+} accumulated in vesicles, which appear to be secondary lysosomes under the electron microscope, but this alkalization does not affect the Ca^{2+} accumulated in the ER [9]. In animal cells, unlike the ER, which is a single organelle, the acidic compartment consists of different organelles, such as Golgi apparatus, lysosomes, secretory granules and endosomes. However, although they all accumulate Ca^{2+} in resting conditions, the molecular mechanism involved appears to be different to SERCA pump, which is involved in Ca^{2+} loading of the ER, because the Ca^{2+} accumulating mechanisms of acidic compartments are insensitive to thapsigargin [10]. It is clear now that these acidic compartments play a key role in cell survival and other cell functions. However, we need to know how much Ca^{2+} is actually released from these acidic compartments and what is the nature of the interaction between these acidic compartments and the ER to fully understand Ca^{2+} regulation by these compartments and the role played by these Ca^{2+} fluxes in cell physiology, particularly in insulin signaling, insulin resistance state and diabetes.

2.1. Acidic compartments require a bafilomycin-sensitive, vacuolar-type H^+ pump to accumulate Ca^{2+}

In animal cells, these acidic Ca^{2+} stores exhibit Ca^{2+} uptake mechanisms that strongly depend on the activity of a vacuolar type of proton pump (V-H^+-ATPase) that is specifically and potently blocked Bafilomycin A1 [11]. It has been suggested that this acidic environment, generated by the proton pump, is then used by a Ca^{2+}/H^+ exchanger to accumulate Ca^{2+} in these organelles using the proton gradient [12]. However, mammalian cells do not seem to express an homologue of this Ca^{2+}/H^+ exchanger; therefore, it is has been proposed that a coupled mechanisms between two exchangers, i.e. Na^+/H^+ and Na^+/Ca^{2+} exchangers might supply the absence of Ca^{2+}/H^+ exchanger but at the end they have a similar result [7].

The Golgi apparatus has an acidic pH of around 6.4 and bafilomycin A1 increases this pH to 7.4 [13]. The Golgi apparatus is a very complex stack of membrane structures divided in three main sections, i.e. cis, medial and trans. Cis section is closer to the ER and accordingly, this part of Golgi presents both IP_3Rs and SERCA pumps, as ER does [14]. Therefore the Ca^{2+} loading of this section is sensitive to thapsigargin. The Trans Golgi accumulates Ca^{2+} using SPCA Ca^{2+} pump, which is molecularly different to SERCA pump and is not inhibited by thapsigargin or CPA [15]. Ca^{2+} uptake in the Medial region presents mix characteristics since it is the combination of the mechanisms present in Cis and Trans regions [14]. It is not clear whether the acidic pH of Golgi Apparatus plays any role in the accumulation of Ca^{2+} by this organelle or Ca^{2+} accumulation and acidic pH are unrelated conditions.

Lysosomes are an heterogeneous vesicular organelles that typically are among the most acidic cellular structures and also the main degradative compartment in eukaryotic cells [16]. This organelle mediates a wide variety of functions, for instance autophagy, a process were cells recycle their components or can also result in cell demise for either homeostatic purposes or degenerative processes [17]. Lysosomes have an extremely low intraluminal pH between 4 and 5.0 which is partially alkalinized (6.5) by inhibiting V-type H^+ pump with bafilomycin A1 [18,19]. One approach to study Ca^{2+} regulation by lysosomes was to expose macrophages to fura-2-dextran to load lysosomes with this Ca^{2+}-sensitive dye by endocytosis. This maneuver revealed that the free $[Ca^{2+}]$ in the lumen of lysosomes was extremely high, of 1 mM, which was totally depleted by incubation with bafilomycin A1 [19]. A different approach to corroborate that lysosome organelle is an internal Ca^{2+} store resulted in an unsettling picture. This approach targeted genetically encoded Ca^{2+} indicators to lysosomes using LAMP1 protein that has been used as a protein marker of lysosomes. LAMP1-YCaM showed that lysosomes can accumulate Ca^{2+} but only in response to an increase in the $[Ca^{2+}]_i$; additionally, neither bafilomycin A1 nor GPN, a substrate for cathepsin C that produces rupture of lysosomes by osmotic swelling, reduced the luminal lysosomal $[Ca^{2+}]$ indicated by LAMP1-YCaM [20]. Interestingly, LAMP1-YCaM shows that lysosomes accumulate Ca^{2+} that depends on the presence of an acidic pH, although Ca^{2+} accumulation was only transient, for instance in response to histamine. Indeed, the lysosomal Ca^{2+} indicator displays oscillating elevations of intra-lysosomal $[Ca^{2+}]$; however, since there were no simultaneous recording of $[Ca^{2+}]_i$ it is difficult to conclude whether histamine-induced oscillations of $[Ca^{2+}]_i$ were behind the observed oscillations of the intralysosomal $[Ca^{2+}]$ [20]. Further work is need to establish what is the actual free and total $[Ca^{2+}]$ present in the lumen of lysosomes and whether these organelles releases Ca^{2+} directly to the cytoplasm.

Secretory granules have an elevated free $[Ca^{2+}]$ of approximately 60 μM [21] or 25 μM [22] and this value is sensitive to changes in the intravesicular pH, which is rather acidic (pH 5.5). Although alkalization of secretory granules with NH_4Cl reduces the free luminal $[Ca^{2+}]$; apparently, this effect is due more to an increase in the buffering capacity of the intraluminal proteins than Ca^{2+} release by the granule [21]. The buffering capacity of the secretory granules involves proteins such as chromogranin, among others. These proteins explain why the total $[Ca^{2+}]$ inside a secretory granule can be in the mM range. Secretory granules can release the stored Ca^{2+} in response to IP_3 by a complex mechanism that involves the transient elevation of the intraluminal $[Ca^{2+}]$ [22] and apparently, it could also involve Orai channels [21] that typically participate more in the store operated Ca^{2+} entry at the plasma membrane.

Endosomes are vesicles that have engulfed the external medium and accordingly should be bringing large amounts of Ca^{2+} inside cells; however, loading Ca^{2+} indicators by endocytosis to study the fate of the Ca^{2+} that has been internalized shows that these vesicles release their Ca^{2+} cargo rather quickly in a correlative manner with their acidification [23]. In other words the more acidic the endosome becomes the less Ca^{2+} can be detected inside the organelle.

Moreover, acidic Ca^{2+} stores besides releasing Ca^{2+} are also involved in buffering the Ca^{2+} that has been released from the ER. Indeed, different approaches to eliminate the Ca^{2+} buffering activity of acidic stores, such as the addition of bafilomycin A1, incubation with GPN to induce osmotic lysis of lysosomes or vacuolin, to increase the size of lysosomes and allegedly decrease the number of contact sites with ER, resulted in higher $[Ca^{2+}]_i$ responses induced by carbachol in HEK cells [24]. These data suggest that lysosomes are in close contact with ER and that their Ca^{2+} uptake mechanism are rapid enough to partially buffer the Ca^{2+} released by IP_3Rs.

2.2. Acidic compartments are endowed with different type of Ca^{2+} permeable channels (TRPM2, TRPML and TPC)

It appears then, that acidic intracellular Ca^{2+} stores particularly lysosomes, are emerging as new players in the Ca^{2+} signaling