Cell Calcium 55 (2014) 346–354

Contents lists available at ScienceDirect

Cell Calcium

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

Role of acidic stores in secretory epithelia

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ARTICLE INFO

Article history:
Received 20 January 2014
Received in revised form 5 April 2014
Accepted 8 April 2014
Available online 18 April 2014

Keywords:
Calcium
Pancreas
Acidic store
Ethanol
CRAC
Calmodulin

ABSTRACT

There is growing evidence that intracellular calcium plays a primary role in the pathophysiology of the pancreas in addition to its crucial importance in major physiological functions. Pancreatic acinar cells have a remarkably large amount of Ca^{2+} stored in both the endoplasmic reticulum (ER) and the acidic stores. The vast majority of the classical ER Ca^{2+} store is located in the basal part of the acinar cells with extensions protruding into the apical area, however, the acidic stores are exclusively located in the secretory granular area of the cells. Both types of Ca^{2+} store respond to all three intracellular Ca^{2+} messengers – inositol trisphosphate ($InsP_3$), cyclic-ADP-ribose (cADPR) and nicotinic acid adenine dinucleotide phosphate (NAADP). The two stores interact with each other via calcium-induced calcium release; however, they can be separated using pharmacological tools. The ER relies on sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) that can be blocked by the specific inhibitor thapsigargin. The acidic store requires a low pH that can be modified by blocking vacuolar H^+-ATPase.

The acidic store is particularly important for pathological processes in the pancreas. Acute pancreatitis is initiated as a result of calcium overload in the apical pole, which leads to trypsinogen activation; two major causes are gall bladder stones and excessive alcohol consumption. Excessive Ca^{2+} release from the acidic stores plays a major role in both scenarios; however NAADP-induced calcium release from acidic stores is particularly important for bile-induced pancreatitis. Cell-permeable calmodulin (CaM) activators such as CALP3 boost the natural protective effect of CaM by inhibiting excessive calcium release from the internal stores through inositol trisphosphate ($InsP_3R$) and ryanodine receptors (RyR). Alternatively calcium overload can be dramatically reduced by inhibiting Ca^{2+}-release-activated Ca^{2+} (CRAC) currents that are required to reload the internal stores and therefore provide effective protection against the major triggers of acute pancreatitis.

1. Introduction

Acinar cells in the secretory epithelia produce substantial amounts of digestive enzymes synthesised as proenzymes and packaged into the secretory (zymogen) granules [1]. Studies of these very powerful exocrine cells have led to many important findings in the field of Ca^{2+} signalling, including the demonstration of calcium release from internal stores in response to stimulation, followed by calcium influx from the interstitial fluid in submandibular glands [2]. Research using pancreatic acinar cells opened many new chapters in Ca^{2+} signalling, including the discovery of the role of $InsP_3$ as an intracellular Ca^{2+} messenger [3], quantal Ca^{2+} release in response to hormone stimulation [4], the polarised distribution of Ca^{2+} channels [1,2,5–9]. The location of the calcium store in the ER has been suggested in the early work on salivary glands [2] and later it has been shown that $InsP_3Rs$ channels are located in the ER [9], including the apical area of acinar cells [10,11]. Most interestingly, however, it was found that $InsP_3$ can release Ca^{2+} from a different organelle containing a vacuolar H^+-ATPase [12]. This was quickly followed up by the discovery of Ca^{2+} release from bovine adrenal medullary secretory vesicles [13]. Our work using isolated pancreatic zymogen granules with measurements carried out both inside and outside a single granule has demonstrated directly [14] that $InsP_3$ and cADPR can release Ca^{2+} from this organelle (Fig. 1). Finally, a study using tracheal goblet cells has confirmed that secretory granules can release Ca^{2+} in response to $InsP_3$ [15].

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http://dx.doi.org/10.1016/j.ceca.2014.04.002
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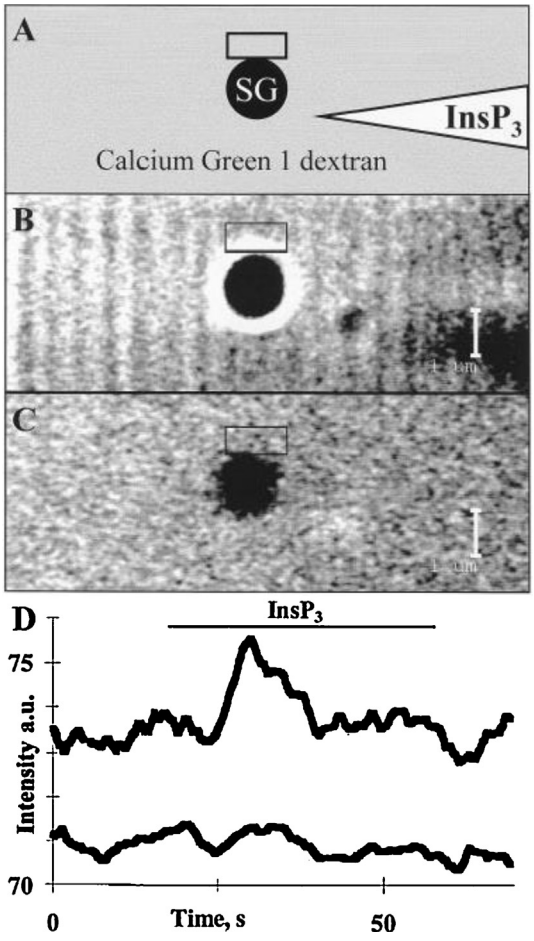

Fig. 1. $InsP_3$ evokes an increase in $[Ca^{2+}]$ concentration in the immediate vicinity of a single isolated secretory granule (modified from [14]). (A) Schematic diagram of the experiment shown in (B–D). $InsP_3$ has been applied ionophoretically using a pipette filled with $InsP_3$. Fluorescence of Calcium Green dextran was recorded from the area near the single isolated granule placed in the extra-granular solution and from the area 15 μm away from the granule. (B) Transparent light image of the single granule and a shade of ionophoretic electrode (on the right). (C) Fluorescence of Calcium Green dextran of the same field shown in (B). Box represents region of interest next to granule for intensity measurements (shown in (D), upper trace). Control box (lower trace in (D)) is 15 μm away from the granule. (D) Fluorescence changes near the granule (top trace) and 15 μm away (lower trace) during experiment. Injection of $InsP_3$ causes rapid elevation of fluorescence intensity of Calcium Green dextran near the granule, while no changes occur in the control region (lower trace).

2. Acidic calcium stores in pancreatic acinar cells

It was reported more than 25 years ago that pancreatic acinar cells contained several types of intracellular Ca^{2+} stores [16,17], which suggested that some of them were acidic. Secretory granules (ZG) have been the main candidate for the acidic stores (AS) because of the high density of ZGs in the secretory granular area and the high calcium content. Several laboratories including our own have shown that $InsP_3$ induces Ca^{2+} release from isolated ZGs and ZGs in intact cells [14,18]. Similar findings have also been made in tracheal goblet cells [15] and mast cells [19]. Our data [14] show that single isolated ZGs respond to $InsP_3$ application by reducing internal free Ca^{2+} and increasing (in separate experiments) free Ca^{2+} concentration in close vicinity of the single granule, measured with Calcium Green dextran (Fig. 1). The control area was 15 μm away from the granule and did not show any change in fluorescence [14].

A very large part of the AS in pancreatic acinar cells is colocalized with ZGs, however, other acid organelles like endosomes and lysosomes can also participate in intracellular Ca^{2+} signalling [20,21]. Ca^{2+} uptake in the AS relies on the bafilomycin-sensitive vacuolar H^+-ATPase [12] and probably the Ca^{2+}/H^+ exchanger [1]. Bafilomycin A1 can be used to block Ca^{2+} uptake into the AS and effectively empty the AS of the available Ca^{2+} content [21,22].

3. The AS interacts with the ER in the apical area

Ca^{2+} release from ZGs could explain local Ca^{2+} spikes in the apical area [21,23,24], however, the ER is also responsible for these. In fact, it is likely that a major proportion of apical Ca^{2+} spikes are due to Ca^{2+} release from ER elements [25] in the secretory granular area (Fig. 2). Co-staining of cells with the ER specific dye ER-Tracker Blue-White and the acidic dye LysoTracker Red shows (Fig. 2A) that there is a substantial amount of ER which is in very close contact with the acidic organelles and ZGs in the apical part of the cell, though less than there is in the basal part. Fig. 2B demonstrates high resolution ER staining in the apical part of pancreatic acinar cells (fluorescent thapsigargin) which confirms that thin protrusions of the ER are present and appear to surround ZGs [25]. ER strands in the apical part of cells receive Ca^{2+} from the larger basal part by diffusion through the lumen because the ER is functionally connected, allowing the diffusion of Ca^{2+} inside the ER throughout the cell [26–30]. The Ca^{2+} buffer capacity in the ER is much lower than in the cytosol, therefore, Ca^{2+} diffuses more easily in the ER lumen [31]. Hence, both the ER and AS are important for Ca^{2+} release in the apical region and for reloading of the Ca^{2+} stores in exocrine cells [32]. Idea of interaction between the AS and the ER has been later proposed as a major mechanism for converging different intracellular messenger pathways [33,34], including bidirectional signalling [35].

4. Acidic store is sensitive to $InsP_3$, cADPR and NAADP

The AS are known to respond to all three intracellular Ca^{2+} messengers in pancreatic acinar cells [1,36,37]. The AS is colocalised mostly with the ZGs. They have much larger volume than other acidic organelles, as well as containing substantially more total Ca^{2+} content [38]. The accumulation of high Ca^{2+} inside the AS depends on a bafilomycin-sensitive vacuolar H^+-ATPase [12,21] and Ca^{2+}/H^+ exchanger [1], while in the early endosomes a high Ca^{2+} content is efficiently used to exchange for H^+ and as a result they quickly acidify [20].

The AS in pancreatic acinar cells has been shown to release Ca^{2+} in response to not only $InsP_3$ and cADPR [14] but also to NAADP [21,33], as shown in Fig. 3A. Using the two-photon permeabilization technique it was shown that in pancreatic acinar cells, the Ca^{2+} release induced by the messenger NAADP is primarily through the RyRs. This is valid for both Ca^{2+} stores, the ER and the AS [21,22]. It is believed that the AS and the ER contain the same types of intracellular Ca^{2+} release channels, $InsP_3$Rs and RyRs. Inhibitors of RyRs abolish the responses to NAADP [21,22]. We have estimated the averaged responses to Ca^{2+} releasing messengers from the AS and found them to be much smaller than the responses from the ER in the whole cell (Fig. 3B). Either of the messengers ($InsP_3$ or cADPR or NAADP) can release about two thirds of the ER content (as compared to thapsigargin responses). The responses to either of the messengers ($InsP_3$ or cADPR or NAADP) from the AS are about 10 times less than the amount that can be released by the calibrations with ionomycin–nigericin–high EGTA [21]. Calibrations also show that in the whole cell the acidic store is approximately half the size of the ER [21]. However, in the secretory granular area there is less difference in the sizes of the stores and the AS is larger than the ER [21]. Calibrations also show that the free Ca^{2+} concentration in the AS is approximately twice that in the ER.