Calcium signalling in and around the nuclear envelope

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Abstract

We have compared calcium mobilization by Ins(1,4,5)P₃ (IP₃), cADP-ribose (cADPR) and nicotinic acid-adenosine dinucleotide phosphate (NAADP) from the envelope of isolated nuclei with the calcium signalling in intact isolated pancreatic acinar cells. Ca²⁺ uptake and release were studied with calcium-sensitive fluorescent probes. In the present study, we have shown that all calcium messengers induce Ca²⁺ release from the nuclear envelope. Pre-treatment of nuclei with thapsigargin completely abolished the responses to the calcium messengers, indicating that Ca²⁺ stores in isolated nuclei are thapsigargin-sensitive. Using different pharmacological tools, we show that Ca²⁺ release from pancreatic nuclei is unlikely to occur from stores other than those with endoplasmic reticulum characteristics. We conclude that all three calcium messengers can release Ca²⁺ from pancreatic acinar nuclear stores, as previously shown for IP₃ and cADPR. It would appear that NAADP releases Ca²⁺ from the same IP₃- and cADPR-sensitive stores with endoplasmic reticulum characteristics.

The mechanisms involved in shaping nuclear calcium responses

Polarized secretory cells represent an interesting example of nuclear calcium handling. In these cells, the calcium sequestering organelles fulfil a dual role in calcium signalling. For some hormonal or neurotransmitter inputs, these organelles serve as a barrier, preventing calcium signals from entering the nucleoplasm; for others, the same organelles serve as the amplifiers of calcium release. Studies made by our group are focused on pancreatic acinar cells, which provide a clear example of structurally and functionally polarized epithelial cells. The apical part of this cell is mainly occupied by secretory granules. The basal area contains the nucleus and a major part of the endoplasmic reticulum (ER). There are also projections of ER to the apical part of the cell [1,2]. Small physiological doses of the secretagogues acetylcholine (ACh) and cholecytokinin produce calcium signals mainly concentrated in the secretory region of this cell [3,4]. Similar responses were also found during fast applications of high concentrations of ACh [5]. During localized calcium transients, a very sharp calcium gradient of a few hundred nanomoles per micrometer are found along the axis connecting the secretory granule region and the nucleus [5]. Surprisingly, such substantial gradients then dissipate without calcium entering the nucleoplasm. These data clearly indicate a very efficient mechanism that protects the nucleoplasm against unwanted calcium signals. However, what is the nature of this mechanism? Our studies indicate that mitochondria are preferentially concentrated on the interface of the apical and basal parts of the cell forming a perigranular mitochondrial belt. Studies using different mitochondrial inhibitors clearly indicate that these organelles have an important role in preventing the spread of calcium signals from the apical region to the basal part of the cell and to the nucleoplasm [6–9]. More recently, we have identified two additional groups of mitochondria: a subplasmalemmal group and, importantly, a perinuclear group. The function of the perinuclear group was clearly demonstrated in experiments with local intranuclear uncaging of caged calcium (nitrophenyl-EGTA). In these experiments, the sequestration of calcium by mitochondria was monitored using Rhod-2, a fluorescent calcium indicator that preferentially concentrates in the mitochondria (when loading occurs by incubation with the acetoxymethyl ester of this indicator). The intranuclear calcium uncaging revealed an elevated calcium concentration in a group of mitochondria that are adjacent to the nuclear envelope, whereas the rest of the mitochondria were unaffected. Practically all pancreatic acinar cells had mitochondria positioned close to the nuclear envelope and some cells had the nucleus completely surrounded by mitochondria. These perinuclear mitochondria could provide further protection for the nucleus against unwanted calcium signals. Indeed, it has been found that, even when the calcium signals invade the basal part of the cell, the nuclear response could be both delayed [10] and smaller in amplitude than in the rest of the basal region [5]. Importantly, recent experiments with mitochondrial uncouplers demonstrate that this difference between the nuclear region and the rest of the basal cytosol disappears after inhibition of mitochondrial function [6,11]. The nucleus in pancreatic acinar cells is surrounded by strands

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Abbreviations used: ACh, acetylcholine; cADPR, cADP-ribose; ER, endoplasmic reticulum; IP₃, inositol 1,4,5-trisphosphate; NAADP, nicotinic acid-adenine dinucleotide phosphate

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of rough ER, which contains calcium pumps and provides a further barrier for calcium [1,12].

During calcium responses in the secretory region of the cell, a proportion of calcium is inevitably lost due to calcium extrusion. This is particularly relevant in pancreatic acinar cells that possess a high density of plasma-membrane calcium pumps that are located mainly in the apical part of the cell surface [13,14]. To generate a sustained pattern of localized apical calcium oscillations, calcium has to be transferred from the basal part of the cell (facing the interstitial fluid and blood) to the apical part of the cells, without triggering a calcium rise in the nucleoplasm and the basal cytosol. This seemingly insurmountable task is possible due to intraorganellar calcium transfer. Our experiments with focal reloading [15] and local calcium uncaging in the ER lumen [16] indicate that pancreatic acinar cells have developed an ingenious mechanism that allows the loading of apical calcium stores by ‘tunnelling’ calcium from the basal part of the cell through the ER lumen. Calcium enters the cell through the store-operated calcium influx channels of the basal membrane and is rapidly taken into the ER lumen due to the activity of ER calcium pumps. Because of the relatively low calcium-binding capacity of the ER [15], calcium moves rapidly through the ER lumen [2] to supply the ER terminals in the secretory region, bypassing the nucleoplasm and the rest of the basal cytosol.

Although local apical signalling is the dominant form of calcium signalling in this cell type, the global calcium signals that invade the nucleus are also triggered by physiological concentrations of agonists. These relatively infrequent events are probably necessary to co-ordinate the secretion with translation and transcription of secretory proteins, processes that occur in the basal part of the cell. In our studies, we have demonstrated the calcium-dependent accumulation of calmodulin in nuclei, a process that could be important for the regulation of gene expression [17]. The global calcium signals originate in the apical part of the cell and then propagate to the basal region as a calcium wave, mediated by calcium-induced calcium release (CICR) [18]. During such global calcium signals, the function of the perinuclear ER changes from calcium sequestration to calcium release. The perinuclear ER indeed possesses a number of interesting calcium-releasing mechanisms.

Two families of intracellular Ca$^{2+}$-release channels, namely Ins(1,4,5)$P_3$ (IP$_3$) and ryanodine receptors, have been well described in the main intracellular calcium store, the ER. They are regulated by two intracellular messengers, IP$_3$ and cADP-ribose (cADPR) respectively. More recently, another molecule has been identified as a potent Ca$^{2+}$-releasing agent: nicotinic acid–adenine dinucleotide phosphate (NAADP), a molecule derived from β-NADP [19–21]. The molecular identity of the NAADP receptor is still obscure. Pancreatic acinar cells were the first mammalian cell type in which NAADP was shown to mobilize Ca$^{2+}$ from an intracellular store. Other studies, including work on pancreatic acinar cells, have suggested functional interactions between the Ca$^{2+}$-release pathways controlled by NAADP, IP$_3$ and cADPR [22].

**Calcium signalling in isolated nuclei**

We have investigated the mobilization of Ca$^{2+}$ stores by IP$_3$, cADPR and NAADP in the nuclear envelope of single isolated mouse pancreatic acinar nuclei. The advantage of this model is that isolated nuclei contain the nuclear envelope and some of the perinuclear ER, but are devoid of other cellular organelles, thus simplifying the interpretation of the experimental results. The calcium content of the nuclear envelope can be monitored using low-affinity calcium indicators loaded in the acetoxyethyl ester form. The distribution of such indicators in the pancreatic acinar nuclei is similar to the distribution of ER markers. There is no staining of the nuclei with mitochondrial markers or markers for acidic organelles. The addition of IP$_3$ or cADPR to the nuclei induces transient decreases of the calcium content of the envelope. Application of NAADP also triggers a transient calcium release from the nuclear envelope of the isolated nuclei. In our previous studies on isolated liver nuclei, we found that IP$_3$ and cADPR release calcium from the nuclear envelope [23]. These findings suggest that the presence of IP$_3$ and cADPR channels is a general property of the perinuclear ER membrane. IP$_3$ and cADPR, in micromolar concentrations, induce calcium release, whereas NAADP, already at submicromolar concentrations, triggers calcium release from the perinuclear ER. The recovery phase of IP$_3$-cADPR- and NAADP-induced calcium transients in the ER lumen is ATP-dependent. In ATP-containing solutions, responses to the messengers show complete recovery, but there are no signs of recovery in the absence of ATP.

Using isolated nuclei, we have also studied the interaction of IP$_3$, cADPR and NAADP. The interplay of these messengers has been reported previously in patch-clamp studies of pancreatic acinar cells [22,24]. First, we used caffeine to inhibit IP$_3$ receptors in isolated pancreatic nuclei. We found that caffeine itself can induce calcium release from isolated nuclei. This effect is well known for many cell types, but intact pancreatic acinar cells do not respond to high doses of caffeine [10]. We assume that a cytosolic factor, which is present in the cytoplasm of intact pancreatic acinar cells and which is missing in the preparation of nuclei, is responsible for inhibition of the effect of caffeine. In the nuclei that are pre-incubated with caffeine, NAADP can induce calcium release similar to the responses in the control untreated nuclei. cADPR also releases calcium in the continuous presence of caffeine, which is consistent with the studies on intact pancreatic acinar cells [10]. Caffeine, however, completely inhibits IP$_3$-induced calcium release from the nuclear envelope. The ability of caffeine to inhibit IP$_3$ receptors was also reported for a number of other cell types [25]. The main conclusion from these experiments is that cADPR- and NAADP-induced calcium release could still be triggered in the presence of caffeine, whereas the IP$_3$-induced release is inhibited. It is known that ryanodine receptors can be blocked by the plant alkaloid ryanodine when applied at high concentrations (approx. 100 µM) [26]. In our experiments, high concentrations of ryanodine did not affect...
IP₃-induced responses, but completely prevented caffeine-induced calcium release. In the presence of ryanodine, NAADP, as well as cADPR, was unable to induce calcium release. These experiments suggest that NAADP and cADPR most probably interact with the ryanodine receptor and do not require facilitation by the IP₃ receptors to trigger the calcium response in isolated nuclei. We cannot exclude that NAADP activates a distinct Ca²⁺ release channel [21,27], which requires interaction with a ryanodine receptor to induce a measurable loss of calcium in the perinuclear ER. Our data are, however, explainable without postulating the presence of a distinct NAADP receptor Ca²⁺ channel (which, so far, has not been identified in any cell type) in isolated nuclei.

Using fluorescent calcium-sensitive probes linked to dextran, we were also able to demonstrate that IP₃, cADPR and NAADP induce Ca²⁺ responses in the nucleoplasm of isolated pancreatic acinar nuclei. Experiments where addition of a high Ca²⁺ concentration is followed by removal of Ca²⁺ show that the nuclear-pore complexes are permeable to Ca²⁺ even after depletion of the nuclear-envelope calcium stores in response to second messengers. This confirms our previous results obtained using isolated liver nuclei [23] and explains the recovery phase of messenger-induced transients.

Similar to other preparations [22,28], application of a high concentration of NAADP completely inhibits NAADP-induced Ca²⁺ release, but the nuclei are still able to respond to IP₃ or cADPR, demonstrating that IP₃ and ryanodine receptors are not affected by the high dose of NAADP.

Pre-treatment of nuclei with thapsigargin [a sarcoplasmic/ER Ca²⁺-ATPase (SERCA) pump inhibitor] completely abolishes the responses to NAADP, indicating that NAADP-sensitive Ca²⁺ stores depend on thapsigargin-sensitive pumps for calcium uptake. Pre-treatment of nuclei with brefeldin A, nigericin or bafilomycin A1 cannot abolish the NAADP-induced Ca²⁺ release, suggesting that NAADP-sensitive Ca²⁺ release from pancreatic nuclei is unlikely to occur from stores other than those with ER characteristics.

Our data suggest that nuclei are important calcium-releasing organelles. Calcium is pumped into the nuclear envelope by powerful thapsigargin-sensitive calcium ATPases. The inner nuclear membrane contains channels that can release calcium directly into the nucleoplasm when stimulated by a number of calcium-releasing messengers (IP₃, cADPR or NAADP).

References

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