Calcium imaging in intact mouse acinar cells in acute pancreas tissue slices

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With the increasing number of patients suffering from diabetes mellitus, pancreatitis, and pancreatic cancer, understanding the normal and pathologic physiology of the pancreas is becoming increasingly important (1). The physiology and pathophysiology of the exocrine pancreas are closely related to changes in the intracellular Ca^{2+} concentration ($[Ca^{2+}]i$ concentration) of the acinar cells. Thus, understanding the regulatory mechanism of $[Ca^{2+}]i$ concentration may lead to new treatments for diabetes mellitus, pancreatitis, and pancreatic cancer. Ca^{2+} is liberated from intracellular stores (the endoplasmic reticulum and acid Ca^{2+} pools) into the cytosol after stimulation by neurotransmitters and hormonal secretagogues, for instance, acetylcholine (ACh) and cholecystokinin (CCK) (2). Under physiological conditions, the increase in $[Ca^{2+}]i$ concentration is mainly confined to the apical region in the form of short-lasting and repetitive local signals responsible for exocytosis and acinar fluid secretion. Eventually, the secretagogue stimulus is sufficient to increase $[Ca^{2+}]i$ concentration globally toward the basal cell pole, affecting ion transport, protein synthesis, and cell metabolism (3). Sustained elevation in $[Ca^{2+}]i$ concentration evoked by pathological agents, such as bile acids, fatty acids, and non-oxidative alcohol metabolites (fatty acid ethyl esters), leads to bioenergetic collapse of the acinar cell, resulting in inappropriate intracellular trypsin and nuclear factor-κB activation, cytoskeletal damage, mitochondrial dysfunction, vacuolization, and necrosis, causing cell injury and acute pancreatitis (4). The main studies to understand the intracellular mechanisms responsible for acinar cell enzyme synthesis and secretion have been performed on cell lines, freshly isolated acinar cells, and isolated pancreatic acini (5). The isolation protocols used in these methods involve digestion by enzymes, a process that results in structural and functional changes (6). To overcome these drawbacks and to allow the study of exo- and endocrine cells in a more natural setting, which could lead to more meaningful results, the acute pancreas tissue slice technique was introduced in 2003 in analogy to successful similar approaches in other tissues (7,8). The main advantage of this approach is the short preparation time without the need for overnight cultivation and enzyme degradation, with initial slices available for experiments in less than an hour (9).

The main aim of our study was to characterize the responses of acinar cells to stimulation with ACh and to compare them with the responses to cerulein in pancreatic tissue slices, focusing on intercellular and interacinar heterogeneity and coupling.

To verify the viability and morphological integrity of acinar cells in mouse acute pancreas tissue slices following isolation, cutting, and loading of the dye, we performed a set of four different and complementary assessments of their structure and ultrastructure. First, high-resolution imaging of the tissue loaded with the fluorescent Ca^{2+} sensitive dye (Oregon Green 488 BAPTA-1 or Calbryte 520 AM) revealed pyramidal-shaped acinar cells distributed concentrically around an intercalate duct or lumen, forming a typical acinus. Second, the slicing procedure hardly affected the viability of the acinar cells as the majority of the cells appeared viable and only a few cells close to the cutting surface appeared dead on the LIVE/DEAD assay. Third, transmission electron microscopy showed the typical acinar cell ultrastructure. Finally, immunohistochemical staining revealed that the acinar cells abundantly expressed the enzyme amylase, the end product of acinar cells, and stimulation of slices by 0.1 nM cerulein resulted in a significant increase in amylase secretion. Immunofluorescence against the basic helix-loop-helix transcription factor Mist-1 showed that the exocrine pancreas organization and acinar cell identity were maintained in the tissue slices.

We have resorted to functional multicellular confocal imaging of mouse acinar cells [Ca^{2+}]i dynamics to characterize their response to ACh and cerulein in tissue slices. We show that acinar cells respond to ACh stimulation with repetitive [Ca^{2+}]i oscillations up to 1000 nM and to the CCK receptor agonist cerulein up to 100 pM. We demonstrated that increasing the ACh stimulation concentration increased both the frequency and duration of the oscillations. The total activity of cells was assessed by calculating the active time, which indicates the fraction of time occupied by oscillations. Given the modulation of frequency and duration described above, the active time is a combination of both, and the average relative active time increased accordingly with increasing ACh concentration. Finally, to evaluate the regularity of the oscillations, we calculated the coefficient of interoscillation interval variability. A significant decrease in interval variability was observed for the most extreme of the tested concentrations, which showed that oscillation became more regular with increased ACh concentration. To quantify the level of intercellular synchronization, we calculated the average coactivity between pairs of cells. The activity was well synchronized only between cells from the same acinus, while the activity pattern differed considerably in the neighboring acini. Therefore, functional connections, reflecting well-synchronized cellular activity, were established only between cell pairs within the same acini and not between the cells from the neighboring acini. Furthermore, the oscillations from the same acinus were similar in shape. To test whether acinar cells in slices also respond to cerulein, a decapeptide cholecystokinin receptor agonist, and to compare its effect on [Ca^{2+}]i oscillations with responses to ACh, we stimulated slices with cerulein at a 10 pM, 100 pM, and 1000 pM concentration (10). The oscillations after simulation with 10 pM cerulein and 100 pM cerulein were the result of repetitive, semiregular cycles of elevated and subsequently decreasing [Ca^{2+}]i levels. The observed oscillations were similar in the same acinus but typically differed between acini, analogous to what we observed in the case of ACh. Quantitative analysis revealed a dose-dependent [Ca^{2+}]i response to cerulein. We observed a significant increase in the duration of oscillations and relative active time at higher cerulein concentrations. In contrast, the frequency of oscillations, their regularity, and the degree of intercellular synchronization were not found to be concentration-dependent.

In conclusion, our results proved that various calcium oscillation parameters depend monotonically on the stimulus concentration and that the activity is rather well synchronized within acini, but not between acini. The acute pancreas tissue slice represents in our eyes a viable and reliable experimental approach for the evaluation of both intra- and intercellular signaling characteristics of acinar cell calcium dynamics. It can be utilized to assess many cells simultaneously with high spatiotemporal resolution, thus providing an efficient and high-yield platform for future studies of normal acinar cell biology, pathophysiology, and screening of pharmacological substances.

Literature

1. Parys JB, Bultynck G. Calcium signaling in health, disease and therapy. Biochim Biophys Acta Mol Cell Res. 2018;1865(11 Pt B):1657–9.
2. Manuel Cancela J, Gerasimenko OV, Gerasimenko JV, Tepikin AV, Petersen OH. Two different but converging messenger pathways to intracellular Ca^{2+} release: the roles of nicotinic acid adenine dinucleotide phosphate, cyclic ADP-ribose ,and inositol trisphosphate. EMBO J. 2000 Jun 1;19(11):2549–57.
3. Petersen OH, Tepikin AV. Polarized calcium signaling in exocrine gland cells. Annu Rev Physiol. 2008;70:273–99.
4. Li J, Zhou R, Zhang J, Li ZF. Calcium signaling of pancreatic acinar cells in the pathogenesis of pancreatitis. World J Gastroenterol WJG. 2014 Nov 21;20(43):16146–52.
5. Williams JA, Korc M, Dormer RL. The acti of secretagogues on a new preparation of functionally intact, isolated pancreatic acini. Am J Physiol. 1978;235(5):517–24.
6. Park MK, Lee M, Petersen OH. Morphological and functional changes of dissociated single pancreatic acinar cells: testing the suitability of the single cell as a model for exocytosis and calcium signaling. Cell Calcium. 2004 Apr;35(4):367–79.
7. Speier S, Rupnik M. A novel approach to in situ characterization of pancreatic beta-cells. Pflugers Arch. 2003 Aug;446(5):553–8.
8. Stožer A, Dolenšek J, Rupnik MS. Glucose-stimulated calcium dynamics in islets of Langerhans in acute mouse pancreas tissue slices. PloS One. 2013;8(1):e54638.
9. Marciniak A, Cohrs CM, Tsata V, Chouinard JA, Selck C, Stertmann J, et al. Using pancreas tissue slices for in situ studies of islet of Langerhans and acinar cell biology. Nat Protoc. 2014 Dec;9(12):2809–22.
10. Marciniak A, Selck C, Friedrich B, Speier S. Mouse pancreas tissue slice culture facilitates long-term studies of exocrine and endocrine cell physiology in situ. PloS One. 2013;8(11):e78706.