Ca²⁺ wave propagation in cells of pancreatic acinus reverses after cell dispersion

TOMIO KANNO^{1,2}, YOSHIKI HABARA² and YOICHI SATOH³

¹Department of Physiology, Faculty of Veterinary Medicine, Hokkaido University, Sapporo 060, ²Laboratory of Cellular Metabolism, Department of Molecular Physiology, National Institute for Physiological Sciences, Okazaki 444, and ³Department of Anatomy, Asahikawa Medical College, Asahikawa 078, Japan

ABSTRACT

Digital image analysis with fura-2 was made to investigate early stage of secretagogue-induced [Ca²⁺]_i increase in pancreatic acinar cells. When *isolated acini* dissociated with collagenase were stimulated with 10 pM CCK-8 or 10 nM ACh, the initial rise in [Ca²⁺]_i occurred in the region beneath the basolateral plasma membrane. When the *acinar cells* isolated with collagenase, trypsin and EGTA were stimulated with 1 mM ACh, the rise started at the luminal region. Morphological deteriorations were detected in the latter preparation. Possible causes for the opposite [Ca²⁺]_i gradient may be in procedures of isolation, destruction of acinar constitution, and composition of perfusing solution.

Cytosolic concentration of calcium, [Ca²⁺]_i, functions as an important intracellular signaling mechanism in stimulus-secretion coupling in a large variety of cells including the pancreatic acinar cell. When the pancreatic acinar cell is stimulated with a physiological concentration of CCK, the dominant source of elevated [Ca²⁺]_i may be the influx of extracellular Ca²⁺ (3, 8). Stimulation with pharmacological concentrations of CCK causes intracellular Ca²⁺ release from Ca stores in addition to the Ca²⁺ influx (3, 8). Digital imaging of [Ca²⁺]_i was recently carried out in two laboratories to record spatial and temporal dynamics of [Ca²⁺]_i in the rat pancreatic acinar cells before and after stimulation with the secretagogues (3, 8, 9). There was a fundamental difference between these reports: the secretagogue-induced rise in [Ca²⁺]_i started at the luminal region and propagated to the basolateral region in the *isolated acinar cells* (9), whereas, in the *isolated acini*, the rise was observed always in the region just beneath the basolateral side of plasma membrane followed by propagation to the luminal region (3, 8). Kasai and Augustine (9) gave an explanation for the difference by pointing out the low resolution of two-wavelength ratio imaging used by Kanno *et al.* (8). Hence we improved the

time resolution of imaging (recorded every 0.2 or 0.3 sec), and then the spatial and temporal analysis of [Ca²⁺]_i dynamics was carried out in *isolated acini* and in *isolated acinar cells*.

Pancreatic *acini* were isolated from the pancreas of male Sprague-Dawley rats (200–250 g body weight) according to the collagenase digestion procedure detailed by Hootman *et al.* (5) with slight modifications described in the previous paper (3). The *isolated acinar cells* were prepared by the method of Maruyama (10). After pelleting and rinsing, *acini* or *acinar cells* were resuspended in fresh Ringer solution and transferred onto a Cell-Tak-coated non-fluorescent glass cover slide mounted in a Sykus-Moore chamber. The chamber was then installed on the stage of an inverted microscope (TMD-2, Nikon, Tokyo, Japan). Digital imaging of fura-2 fluorescence was carried out as reported previously (3, 8) with following modifications. Illumination of the cells with UV light at a wavelength of 340 nm or 360 nm was performed using 10 nm band-width interference filter. The images emitted by 340 nm lighting was recorded every 0.2 or 0.3 sec throughout the experiments, and the images emitted by 360 nm lighting was formed only at the beginning and the end of the experi-

Fig. 1

ments. To form the images at sub-second intervals, the fluorescence ratios (F340/F360) were calculated pixel by pixel from the stored data after the experiment assuming a linear decay of fluorescence intensity emitted by 360 nm lighting during the experiment, and converted to [Ca²⁺]_c using the equation described by Grynkiewicz *et al.* (2).

For electron microscopic examination, the acinar cells were fixed in a fixative containing 1.25% glutaraldehyde and 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). After dehydration, they were embedded in Epon 812. Semithin sections and ultrathin sections were cut.

The *isolated acini* selected for study were examined under a differential interference contrast (DIC) microscope (Fig. 1A). The acini consisted of five acinar cells (about 20 μm in diameter), at the center of which the acinar lumen was recognized. In acinar cells, regional morphological differences were also observed: the apical half of the acinar cells contained abundant zymogen granules (ZG region), whereas the basal half appeared clear with few granules (region of endoplasmic reticulum; ER region).

The [Ca²⁺]_c was low and slightly non-uniform in single acinus even in the absence of any secretagogue. When the acinus was continuously stimulated by replacing the standard perfusing solution with the solution containing 10 pM CCK-8, the distribution of [Ca²⁺]_c changed during the course of continuous stimulation. The [Ca²⁺]_c at the region beneath the basolateral plasma membrane began to increase first in a cell located on the left-hand side of the acinus, despite the solution containing CCK-8 was perfused from the bottom to the top of the figures throughout the present experiments. The Ca²⁺

wave then propagated to the ZG region of the cell (Fig. 1D), and the distinct [Ca²⁺]_c gradient was detected from the ER region (1,000–1,750 nM) to the ZG region (650–1,000 nM) (Fig. 2D). Similar results were obtained in 5 other preparations. When the other *isolated acinus* (Fig. 1B) was continuously stimulated with 10 nM ACh, the initial rise in [Ca²⁺]_c was observed at the region beneath the basolateral plasma membrane in a cell located at bottom side of the acinus, followed by gradual propagation to the ZG region of the cell (Fig. 1E). The [Ca²⁺]_c gradient was also demonstrated from the ER region (650–900 nM) to the ZG region (400 nM) (Fig. 2E). Similar results were obtained in 4 other preparations. When a cluster of three *isolated acinar cells* (Fig. 1C) was continuously stimulated with 1 mM ACh, the initial rise in [Ca²⁺]_c was evoked at the center of the cluster, followed by propagation to the peripheral region of the cells (Fig. 1F). The reverse [Ca²⁺]_c gradient was thus formed from the central region (400 nM) to the peripheral region (300 nM) (Fig. 2F). Similar results were obtained in 2 other preparations.

Isolated acini or the cluster of *isolated acinar cells* were examined under a light or an electron microscope (Fig. 3, A, B, C and D). *Isolated acini* perfused without added secretagogues, the acinar cells showed well preserved structure similar to that described earlier in rat pancreas *in vivo* (4) or after vascular perfusion with the standard Ringer solution (7) (Fig. 3A). After about 5 min stimulation with either 10 pM CCK-8 or 10 nM ACh, the acinar cell ultrastructure was unchanged except decreased number of zymogen granules (Fig. 3B). The secretory response to CCK-8, ACh and NaF (an activator of various guanine nucleotide binding proteins) has already been confirmed in perfused acini isolated from rat pancreas (4, 8).

Isolated acinar cells of the cluster showed neither acinar structure nor cell polarity and contained many vacuoles of various sizes (Fig. 3C). Some vacuoles opened to the extracellular environment. Even after the continuous stimulation with ACh at a high concentration, the cells still reserved abundant zymogen granules, electron density of which was heterogeneous and some of which exhibited dual layers (Fig. 3D). Rough-surfaced endoplasmic reticulum was markedly swollen. These morphological features may correlate with 1) the initial increase in [Ca²⁺]_c at the luminal pole, 2) lack of secretory responses to secretagogues, and 3) stimulus-induced increase in membrane capacitance

Fig. 1 DIC images of *isolated acini* and a cluster of three *isolated acinar cells*, and changes in the distribution of [Ca²⁺]_c during continuous stimulation with 10 pM CCK-8, 10 nM ACh, or 1 mM ACh. Before fluorescence imaging, the preparations were examined under DIC microscope (A, B, and C). Images of fluorescence ratios were formed by dividing the 340 nm image after background subtraction by the 360 nm image after background subtraction (D, E, and F) and [Ca²⁺]_c was expressed as pseudocolor. The images (left to right) in D were recorded every 0.3 sec and the images in E and F were recorded every 0.2 sec (see Fig. 2 legend). The secretagogues were applied several seconds or tens of seconds before the recordings of images depicted in these figures.

Fig. 2 Spatial and temporal features of CCK-8- or ACh-induced $[Ca^{2+}]_c$ changes in isolated acini and acinar cells. Schematic diagrams of isolated acini (A and B) or acinar cells (C) depicted in Fig. 1. The time courses of $[Ca^{2+}]_c$ change caused by 10 pM CCK-8 (D) or 10 nM ACh (E) in isolated acinus and by 1 mM ACh (F) in isolated acinar cells were measured in $1 \times 1 \mu m$ areas in the regions just beneath the basolateral plasma membrane (Basal, solid lines) and luminal portion (Luminal, dotted lines) as shown in each schematic diagram. Abscissas indicate the time (in sec) after perfusing solution containing each secretagogue entered the chamber. Sets of images shown in Fig. 1 were selected from the period indicated by thick black bars.

Fig. 3 Light and electron micrographs of pancreatic acinar cells of *isolated acini* without secretagogues (A) or after 5 min stimulation with 10 pM CCK-8 (B), and of *isolated acinar cells* in the cluster without secretagogues (C) and after 5 min stimulation with 1 mM ACh (D). Horizontal bars represent 10 μ m (A and C) and 1 μ m (B and D). A: Clear lumen (L) and cells of acinar structure containing many zymogen granules are visible. B: Zymogen granules (Z), nucleus (N) and other cell organelles show normal ultrastructure. C: Acinar cells around the inconspicuous acinar lumen are vacuolated. They do not retain the exact cell polarity. D: The cell contains many vacuoles of various sizes (V) at the peripheral cytoplasm. Some vacuoles opened to the extracellular environment. Abundant zymogen granules remained even after the vigorous stimulation but were heterogeneous in the electron density.

measured by patch-clamp method (10, 11).

Possible explanations for the differences in secretory response, acinar cell morphology, and cytosolic Ca²⁺ gradients between the *isolated acinar cells* and the *isolated acini* may be in the procedures of isolation and in the composition of solution perfusing the preparations. The *isolated acinar cells* have usually been prepared by sequential digestion with collagenase and trypsin in a Ca²⁺-free Krebs solution containing Ca²⁺-chelating agent, and then perfused with HEPES-buffered Ringer solution containing neither amino acids, nor bovine serum albumin, nor trypsin inhibitor (9, 10). The *isolated acini* have been prepared by digestion with HEPES-buffered solution containing collagenase, trypsin inhibitor, and bovine serum albumin supplement-

ed with amino acids with neither Ca²⁺-chelating agent nor trypsin (3, 5). Preservation of cellular polarity and intercellular connection with gap junctions and acinar constitution may be of importance for maintaining physiological functions of pancreatic acinar cells *in vitro*. We are still far from a complete molecular understanding of plasma membrane protein sorting in epithelial cells (6). However, the present results afford the direct evidence that the rate of membrane flow (1) may greatly be changed by the procedures of isolation, destruction of acinar constitution, and composition of extracellular environment.

Received 2 October 1991; and accepted 15 October 1991

REFERENCES

1. BENNETT H. S. (1969) The cell surface: components and configurations. In *Handbook of Molecular Cytology* (ed. LIMA-DE-FARIA A.) North-Holland Publishing Company, Amsterdam, pp. 1295-1319
2. GRYNKIEWICZ G., POENIE M. and TSIEN R. Y. (1985) A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. *J. Biol. Chem.* **260**, 3440-3450
3. HABARA Y. and KANNO T. (1991) Dose-dependency in spatial dynamics of $[\text{Ca}^{2+}]_c$ in pancreatic acinar cells. *Cell Calcium* **12**, 533-542
4. HABARA Y., SATOH Y., SAITO T. and KANNO T. (1990) A G-protein activator, NaF, induces $[\text{Ca}^{2+}]_o$ -dependent $[\text{Ca}^{2+}]_c$ oscillation and secretory response in rat pancreatic acini. *Biomedical Res.* **11**, 389-398
5. HOOTMAN S. R., BROWN M. E., WILLIAMS J. A. and LOGSDON C. D. (1986) Regulation of muscarinic acetylcholine receptors in cultured guinea pig pancreatic acini. *Amer. J. Physiol.* **251**, G75-G83
6. HUBBARD A. L., STIEGER B. and BARTLES J. R. (1989) Biogenesis of endogenous plasma membrane proteins in epithelial cells. *Annu. Rev. Physiol.* **51**, 755-770
7. KANNO T., MATSUMOTO T., MORI M., OYAMADA M. and NEVALAINEN T. J. (1984) Secretin prevents hyporeactive and morphological responses of rat pancreatic acinar cells to stimulation with supraoptimal concentration of cholecystokinin-octapeptide. *Biomedical Res.* **5**, 355-370
8. KANNO T., SAITO T. and YAMASHITA T. (1989) Spatial and temporal oscillation of $[\text{Ca}^{2+}]_i$ during continuous stimulation with CCK-8 in isolated rat pancreatic acini. *Biomedical Res.* **10**, 475-484
9. KASAI H. and AUGUSTINE G. J. (1990) Cytosolic Ca^{2+} gradients triggering unidirectional fluid secretion from exocrine pancreas. *Nature* **348**, 735-738
10. MARUYAMA Y. (1988) Agonist-induced changes in cell membrane capacitance and conductance in dialysed pancreatic acinar cells of rats. *J. Physiol.* **406**, 299-313
11. OSIPCHUK Y. V., WAKUI M., YULE D. I., GALLACHER D. V. and PETERSEN O. H. (1990) Cytoplasmic Ca^{2+} oscillations evoked by receptor stimulation, G-protein activation, internal application of inositol trisphosphate or Ca^{2+} : simultaneous microfluorimetry and Ca^{2+} dependent Cl^- current recording in single pancreatic acinar cells. *EMBO J.* **9**, 697-704