Oscillatory Ca^{2+} Signaling in Somatostatin-Producing Cells From the Human Pancreas

Alf Berts, Yi-Jia Liu, Erik Gylfe, and Bo Hellman

Oscillatory Ca^{2+} signaling was studied in human somatostatin-releasing pancreatic δ cells identified by immunostaining. A ratiometric fura-2 technique was used for measuring cytoplasmic concentrations of Ca^{2+} and Sr^{2+} in δ cells exposed to the respective cation. Rhythmic activity in terms of slow (frequency, 0.1 to 0.4 per minute) oscillations from close to the basal level was seen in the presence of 3 to 20 mmol/L glucose during superfusion with medium containing 2.6 to 5 mmol/L Ca^{2+} or 5 mmol/L Sr^{2}. These oscillations could be transformed into a sustained increase by decreasing extracellular Ca^{2+} or adding 1 mmol/L tolbutamide or 20 nmol/L glucagon. Addition of glucagon to a medium containing 20 mmol/L glucose resulted in the generation of short (< 30 seconds) transients, which disappeared upon exposure to 100 nmol/L of the intracellular Ca^{2+}-adenosine triphosphatase (ATPase) inhibitor thapsigargin. When analyzing small aggregates of islet cells, it became evident that oscillatory activity in δ cells can be synchronous with that in adjacent non–δ cells. It is concluded that secretion of pancreatic somatostatin in man involves Ca^{2+} signaling similar to that regulating the pulsatile release of insulin.

AN INCREASE in the cytoplasmic Ca^{2+} concentration is known to stimulate a number of cellular events, including release of hormones from the endocrine pancreas.[1-3] Taking advantage of the sensitivity offered by time-sharing ratiometric measurements with the fluorescent indicator fura-2, we have demonstrated that insulin-producing β cells have an intrinsic rhythmic activity in terms of glucose-induced slow oscillations of cytoplasmic Ca^{2+}.[4] Similar oscillatory Ca^{2+} signaling was recently found also in islet cells responsible for the pulsatile release of glucagon and somatostatin.[5-7] Using mouse cells, it was shown that glucose-regulated somatostatin release mimics that of insulin with the induction of slow oscillations of cytoplasmic Ca^{2+} suppressible with L-epinephrine.[6]

Measurements of cytoplasmic Ca^{2+} levels in individual islet cells derived from the human pancreas have so far demonstrated oscillations in the insulin-producing β cells.[8] We now present the first report on human somatostatin-producing δ cells. It will be shown that these cells generate both fast and slow oscillations of cytoplasmic Ca^{2+} and that the oscillatory activity can be synchronous with adjacent non–δ cells.

MATERIALS AND METHODS

Preparation of Islet Cells

Human islets of Langerhans were isolated by collagenase digestion at the Central Unit for β-Cell Transplantation, Vrije Universiteit Brussels, (Brussels, Belgium), adhering to a previously described protocol.[8] The islets were taken from three female cadaveric organ donors (34 to 49 years old). Following transport to Uppsala, the islets were cultured at 37°C in an atmosphere of 5% CO_2 in humidified air in RPMI 1640 medium supplemented with 10% fetal calf serum, 100 IU penicillin, 100 μg/mL streptomycin, 10 μg/mL gentamicin, and 5.6 mmol/L glucose. Three to 7 days after isolation, the islets were dispersed into single cells by shaking in a medium deprived of Ca^{2+}. The single cells were then allowed to attach to circular 25-mm cover glass during culture for another 1 to 3 days. Subsequent experimental handling was performed with a basal medium containing 125 mmol/L NaCl, 5.9 mmol/L KCl, 1.2 mmol/L $MgCl_2$, 2.6 mmol/L $CaCl_2$ or 5 mmol/L $SrCl_2$, 0.5 mg/ml albumin, and 25 mmol/L HEPES. NaOH was used to adjust pH to 7.40.

Measurements of Cytoplasmic Ca^{2+} and Sr^{2+}

The cells were loaded with the indicator fura-2 in the presence of Ca^{2+} or Sr^{2+} during an incubation of 30 to 40 minutes at 37°C in a medium supplemented with 1 μmol/L fura-2 acetoxymethyl ester and 3 mmol/L glucose. Cover glasses with the attached cells were then used as exchangeable bottoms of an open chamber placed on the stage of an inverted microscope and superfused at a rate of 0.75 mL/min at 37°C. Thapsigargin, which sticks to plastic, was added directly to the superfusion chamber. The superfusion flow was then interrupted for 2 to 3 minutes to ascertain an effect of the drug.

Since the Sr^{2+} complex of fura-2 has spectral properties similar to those of Ca^{2+},[9] it was possible to measure cytoplasmic concentrations of both ions using dual-wavelength fluorometry according to the principles used by Grynkiewicz et al.[10] Fluorescence emitted at 510 nm was recorded either with a photomultiplier[4] or by an intensified video camera.[11] Autofluorescence was negligible and not compensated for. In accordance with our previous measurements of Sr^{2} in pancreatic β cells,[12] the cytoplasmic concentration of this ion was presented as the 340/380 nm fluorescence excitation ratio.

Identification of δ Cells

After fura-2 analyses, the cells were fixed for 5 minutes with 95% ethanol and identified by immunostaining while in position in the superfusion chamber. Cells lacking immunoreactivity for insulin (biotin-streptavidin technique) and reacting positively to subsequent immunostaining for somatostatin (peroxidase-antiperoxidase technique) were classified as δ cells (Fig 1).

RESULTS

Oscillations of cytoplasmic Ca^{2+} from a basal level (frequency, 0.1 to 0.4 per minute) were seen at glucose concentrations of 3 to 20 mmol/L when single δ cells were superfused with a medium containing 2.6 to 5 mmol/L Ca^{2+} (Fig 2). A similar but more regular rhythmic activity occurred during superfusion with a medium nominally devoid of Ca^{2+} but containing 5 mmol/L Sr^{2+} (Fig 3). Oscillatory signaling was demonstrated in δ cells exposed to 3 to 20 mmol/L glucose in five separate experiments. Due to the small proportion of δ cells

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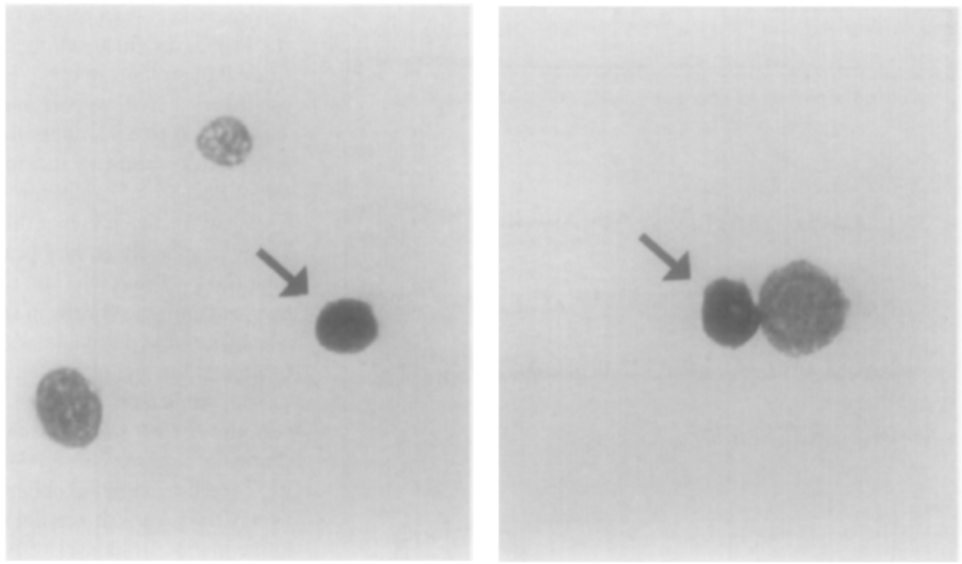

Fig 1. Light-microscopic appearance of isolated pancreatic islet cells first immunostained for insulin and then for somatostatin. Darkly stained δ cells are easily recognized (arrows).

and the restriced availability of human islets, further characterization of oscillatory properties had to rely on single observations. Oscillatory activity was attenuated when extracellular Ca^{2+} was decreased from 5 to 1.3 mmol/L (Fig 2A). Transformation of oscillatory activity into a sustained increase was obtained also after addition of glucagon (Fig 2B) or tolbutamide (Fig 3B). Apart from transforming the slow oscillations into a sustained increase, the addition of glucagon induced numerous superimposed short transients. These transients disappeared after exposure to the intracellular Ca^{2+}-adenosine triphosphatase (ATPase) inhibitor thapsigargin.

Figure 4 illustrates synchronous oscillatory activity in two adjacent non–δ cells and less obvious synchronization with a δ cell within a cluster exposed to 3 mmol/L glucose. However, after elevation of glucose to 20 mmol/L, all three cells oscillated in a coordinated fashion. When glucose was omitted, oscillations tended to fade, but there was an immediate response in all three cells upon subsequent introduction of tolbutamide.

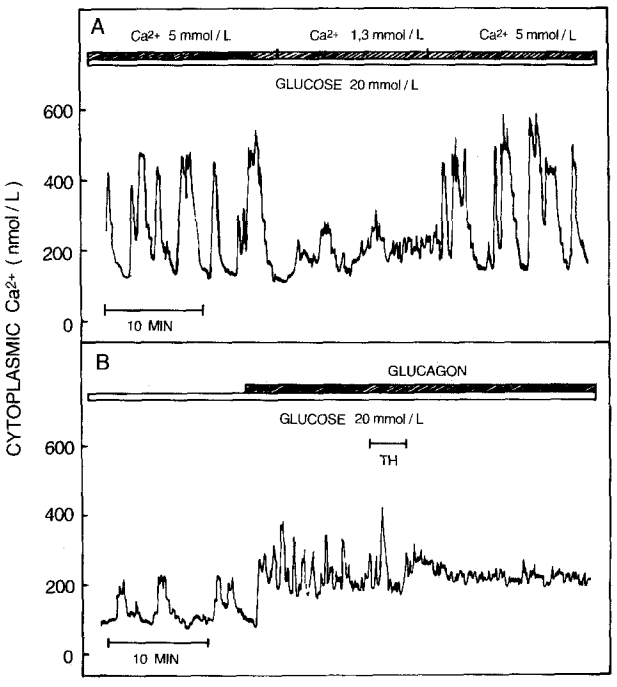

Fig 2. Oscillations of cytoplasmic Ca^{2+} in single δ cells exposed to 20 mmol/L glucose. (A) Results of changing extracellular Ca^{2+} concentration between 1.3 and 5 mmol/L. (B) Slow oscillations in 2.6 mmol/L Ca^{2+} are transformed into a sustained increase with superimposed short transients after addition of 20 nmol/L glucagon. These transients disappear after exposure to 100 nmol/L thapsigargin.

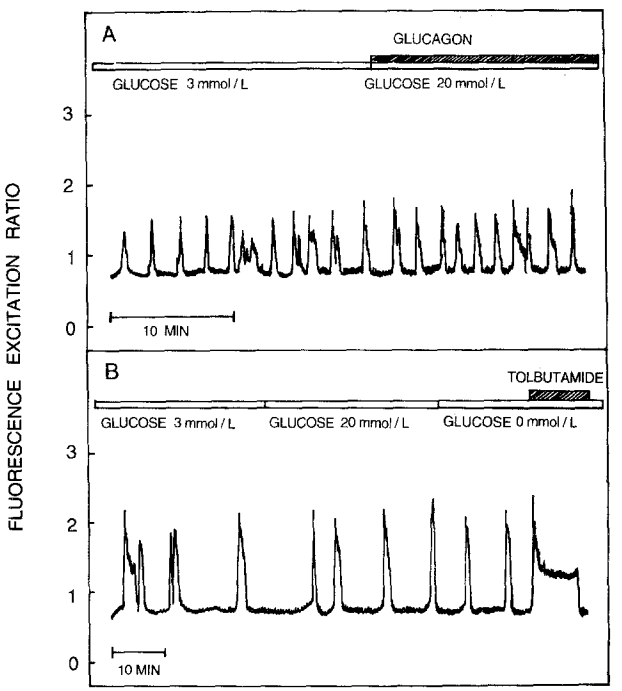

Fig 3. Oscillations of cytoplasmic Sr^{2+} in single δ cells exposed to different concentrations of glucose. (A) Results of combining an increase of glucose to 20 mmol/L with addition of 20 nmol/L glucagon. (B) Oscillatory activity is transformed into a sustained increase by addition of 1 mmol/L tolbutamide.

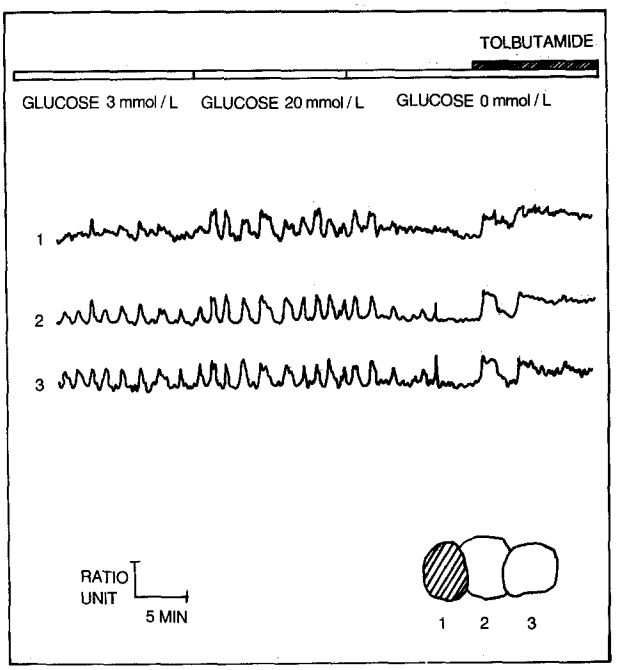

Fig 4. Synchronization of Sr^{2+} oscillations in an islet cell cluster. Variations in cytoplasmic Sr^{2+} are shown for each of 3 cells illustrated in the lower right corner. (▨) Identified as a δ cell by immunostaining for somatostatin.

DISCUSSION

Plasma levels of the major pancreatic islet hormones exhibit characteristic cycles, which seem to correspond to oscillations of cytoplasmic Ca^{2+} in the cells where they are produced.[3-8] Inadequacies in the isolation procedure often result in a disappearance of this Ca^{2+} rhythmicity.[5,13] The requirement for gentle isolation of the islets under conditions of appropriate oxygen supply can best be fulfilled using experimental animals like rats and mice with an extended thin pancreas. Studies of δ cells from the solid human pancreas are also difficult because these cells represent only about 10% of the total number of endocrine cells.[14] Moreover, human δ cells, like those in experimental animals,[15] have elongated cytoplasmic processes, further complicating their successful isolation.[16] In the present study, identification of δ cells was made at the end of the experiments based on both a lack of immunoreactivity for insulin and a positive immunostaining for somatostatin. The fact that Ca^{2+} signaling had to be studied in cells for which the identity was determined at the end of the experiments added to the factors restricting the number of δ cells studied.

Previous investigations have indicated that Sr^{2+} is useful as an analog for Ca^{2+} when demonstrating oscillatory activities in β cells.[12] Although there were no differences between the two cations with regard to the type of oscillation obtained, more stable patterns are generally observed with Sr^{2+}. It was evident from the present studies that prolonged periods of regular oscillatory activity appear more frequently when human δ cells are exposed to Sr^{2+} rather than Ca^{2+}. In addition to the slow oscillations occurring in mouse δ cells exposed to 3 to 20 mmol/L glucose,[5,6] glucagon was now found to induce irregular transients in the presence of the higher sugar concentration. This glucagon effect disappeared after exposure to the intracellular Ca^{2+}-ATPase inhibitor thapsigargin, indicating that δ cells may be similar to β cells in responding to an increase of cyclic adenosine monophosphate with depolarization-dependent mobilization of intracellular calcium.[17]

The similarities between the effects of glucose on the secretion of somatostatin and insulin are apparent from the sigmoidal dose-response relationship,[18] the anomeric specificity,[19] and the inhibition obtained when suppressing the metabolism of the sugar.[18] It was not surprising to observe a parallelism in the glucose responses of islet β and δ cells, since studies in mice[6] have shown that the latter cell type also has adenosine triphosphate (ATP)-sensitive K^+ channels. Since most of the effects of hypoglycemic sulfonylureas can be attributed to closure of ATP-sensitive K^+ channels,[20,21] the observed response to tolbutamide indicates that human δ cells also have such channels. Slow oscillations with the same characteristics as in δ cells from mice[5,6] were now found to be transformed into sustained elevation by decreasing extracellular Ca^{2+} or adding glucagon or tolbutamide. Since each of these measures are known to promote such a transformation also in isolated β cells, the observations provide further support for the involvement of similar ionic events in the regulation of somatostatin and insulin release.

Somatostatin is a potent inhibitor of insulin and glucagon release from pancreatic islets.[19,22] Being located in close relation to both β cells and α cells, it has been suggested that δ cells act as paracrine modulators of insulin and glucagon release.[22,23] It has also been proposed that there is an intraislet paracrine signaling pathway directed from β cells via α cells to δ cells.[24] Microinjection of fluorescent dye has revealed gap junctional coupling between different types of islet cells.[25,26] We now present evidence indicating that oscillatory signaling in human δ cells can be synchronized with adjacent non–δ cells. Since gap junctional communication apparently represents an alternative mechanism for intercellular signaling, it is possible that the interaction between islet cells is different from that expected from the action of the secreted hormones.

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REFERENCES

1. Hermansen K, Christensen SE, Ørskov H: Characterization of somatostatin release from the pancreas. Diabetologia 16:261-266, 1979

2. Niki I, Tamagawa T, Niki A, et al: Glucagon release dependent on and independent of changes in cytosolic Ca^{2+}: Studies using Ca^{2+}-clamped rat pancreatic islets. Biomed Res 7:291-294, 1988

3. Hellman B, Gylfe E, Bergsten P, et al: The role of Ca^{2+} in the release of pancreatic islet hormones. Diabete Metab 20:123-131, 1994

4. Grapengiesser E, Gylfe E, Hellman B: Glucose-induced oscillations of cytoplasmic Ca^{2+} in the pancreatic β-cell. Biochem Biophys Res Commun 151:1299-1304, 1988

5. Berts A, Gylfe E, Hellman B: Ca^{2+} oscillations in pancreatic islet cells secreting glucagon and somatostatin. Biochem Biophys Res Commun 208:644-649, 1995

6. Berts A, Ball A, Dryselius S, et al: Glucose stimulation of somatostatin-producing islet cells involves oscillatory Ca^{2+} signalling. Endocrinology 137:693-697, 1996

7. Berts A, Ball A, Gylfe E, et al: Suppression of Ca^{2+} oscillations in glucagon-producing α_2-cells by insulin/glucose and amino acids. Biochim Biophys Acta 1310:212-216, 1996

8. Hellman B, Gylfe E, Bergsten P, et al: Glucose induces oscillatory Ca^{2+} signalling and insulin release in human pancreatic beta cells. Diabetologia 37:S11-S20, 1994 (suppl)

9. Kwan CY, Putney JW Jr: Uptake and intracellular sequestration of divalent cations in resting and methacholine-stimulated mouse lacrimal acinar cells. Dissociation by Sr^{2+} and Ba^{2+} of agonist-stimulated divalent cation entry from the refilling of the agonist-sensitive intracellular pool. J Biol Chem 265:678-684, 1990

10. Grynkiewicz G, Poenie M, Tsien RY: A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. J Biol Chem 260:3440-3450, 1985

11. Gylfe E, Grapengiesser E, Hellman B: Propagation of cytoplasmic Ca^{2+} oscillations in clusters of pancreatic β-cells exposed to glucose. Cell Calcium 12:229-240, 1991

12. Liu Y-J, Grapengiesser E, Gylfe E, et al: Glucose induces oscillations of cytoplasmic Ca^{2+}, Sr^{2+} and Ba^{2+} in pancreatic β-cells without participation of the thapsigargin-sensitive store. Cell Calcium 18:165-173, 1995

13. Hellman B, Gylfe E, Bergsten P, et al: Pulsatile Ca^{2+} signalling and insulin release, in Flatt PR, Lenzen S (eds): Insulin Secretion and Pancreatic β-Cell Research. London, UK, Smith Gordon, 1994, pp 221-228

14. Rahier J: The diabetic pancreas: A pathologist's view, in Lefebvre PJ, Pipeleers DG (eds): The Pathology of the Endocrine Pancreas in Diabetes. Berlin, Germany, Springer Verlag, 1988, pp 17-40

15. Hellerström C, Hellman B, Petersson B, et al: The two types of pancreatic A-cells and their relation to glucagon secretion, in Brolin SE, Hellman B, Knutson H (eds): The Structure and Metabolism of the Pancreatic Islets. Oxford, UK, Pergamon, 1964, pp 117-130

16. Pipeleers DG, Pipeleers-Marichal MA: A method for purification of single A, B and D cells and for the isolation of coupled cells from isolated rat islets. Diabetologia 20:654-663, 1981

17. Liu Y-J, Grapengiesser E, Gylfe E, et al: Crosstalk between the cAMP and inositol trisphosphate signalling pathways in pancreatic β-cells. Arch Biochem Biophys 334:295-302, 1996

18. Hermansen K: Secretion of somatostatin from the normal and diabetic pancreas. Diabetologia 19:492-504, 1980

19. Leclercq-Meyer V, Woussen-Colle M-C, Lalieu C, et al: Anomeric specificity of glucose-induced somatostatin secretion. Experientia 43:1216-1218, 1987

20. Sturgess NC, Ashford MLJ, Cook L, et al: The sulfonylurea receptor may be an ATP-sensitive potassium channel. Lancet 2:474-475, 1985

21. Garcia-Barrado MJ, Jonas JC, Gilon P, et al: Sulfonylureas do not increase insulin secretion by a mechanism other than a rise in cytoplasmic Ca^{2+} in pancreatic β-cells. Eur J Pharmacol 298:279-286, 1996

22. Taborsky GJ Jr: Evidence of a paracrine role for pancreatic somatostatin in vivo. Am J Physiol 245:E598-E603, 1983

23. Orci L, Unger RH: Functional subdivision of islets of Langerhans and possible role of D-cells. Lancet 2:1243-1244, 1975

24. Samols E, Stagner JI: Islet somatostatin—Microvascular, paracrine and pulsatile regulation. Metabolism 39:55-60, 1990

25. Michaels RL, Sheridan JD: Islets of Langerhans: Dye coupling among immunocytochemically distinct cell types. Science 214:801-803, 1981

26. Meda P, Kohen E, Kohen C, et al: Direct communication of homologous and heterologous endocrine islet cells in culture. J Cell Biol 92:221-226, 1982