FEBS Letters 581 (2007) 4080–4084

^{3}H-serotonin as a marker of oscillatory insulin secretion in clonal β-cells (INS-1)☆

Jude T. Deeney[a,b,*], Robert Bränström[a], Barbara E. Corkey[b], Olof Larsson[a], Per-Olof Berggren[a]

[a] The Rolf Luft Research Center for Diabetes and Endocrinology, Karolinska Institutet, S-171 76, Stockholm, Sweden
[b] Obesity Research Center, Evans Department of Medicine, Boston Medical Center, EBRC 804, 650 Albany St., Boston, MA 02118, United States

Received 19 February 2007; revised 29 May 2007; accepted 20 July 2007

Available online 30 July 2007

Edited by Felix Wieland

Abstract **Serotonin release from preloaded pancreatic β-cells has been used as a marker for insulin release in studying exocytosis from single cells using the amperometric technique. We found that single pancreatic β-cells exhibited oscillations in exocytosis with a period of 1–1.5 min as measured amperometrically by serotonin release. We also show that ^{3}H-serotonin can be used to monitor exocytosis from intact and streptolysin-O permeabilized clonal insulin-secreting cells preloaded with labeled serotonin and that serotonin release correlated with insulin secretion in the same cells. The use of ^{3}H-serotonin provides a real-time indicator of exocytosis from populations of clonal insulin-secreting cells.**

Keywords: Serotonin; Insulin-secreting cell; Exocytosis; Insulin secretion

1. Introduction

Impaired insulin release from the pancreatic β-cell is one of the causative factors of type 2 diabetes and has been intensively investigated over the past four decades. Insulin release has been measured from man and whole animals as well as perfused pancreas and perifused islets, dissociated islets and clonal pancreatic β-cells [1–3].

Exocytosis has been measured using a variety of different techniques in pancreatic islets or β-cells. These techniques include traditional radioimmunoassay [3], and amperometric measurements of insulin release from single pancreatic β-cells using ruthenate-coated carbon fiber electrodes to catalyze the oxidation of the insulin disulfide bonds [4,5]. Due to difficulties in making reliable insulin specific electrodes, a more basic serotonin-sensing carbon fiber is often used to measure exocytosis from cells, which have been preincubated with serotonin for 2–24 h [6,7]. Serotonin is taken up into the β-cell, localizes to the insulin secretory granules [8,9] and is then co-secreted with insulin [10]. Serotonin release from single preloaded pancreatic β-cells has been measured using the amperometric technique and has correlated well to insulin exocytosis as measured by the same technique using the specific insulin sensitive carbon fiber electrode [11].

Exocytosis can be measured radioisotopically during batch incubation experiments. ^{3}H-epinephrine can be loaded into PC12 cells in order to monitor secretion of ^{3}H-norepinephrine from permeabilized cells [12]. ^{3}H-serotonin release has been measured from preloaded clonal insulin-secreting RINm5F cells depolarized with KCl, but no correlation with insulin release was attempted [13]. Here, we show that ^{3}H-serotonin can be loaded into clonal pancreatic β-cells (INS-1) in order to monitor exocytosis from both intact and permeabilized cells validating the use of serotonin as a marker for insulin secretion.

2. Materials and methods

2.1. Animals and preparation of primary cultured β-cells

Pancreatic islets of Langerhans were isolated from a non-inbred colony of adult obese mice (gene code ob/ob) [14]. The mice were killed by decapitation after being fasted for 24-h and dispersed islets were isolated by a collagenase technique [15]. The islets of these mice contain more than 90% β-cells [16]. A cell suspension was prepared as described previously [17], the cells seeded into petri dishes and resuspended in RPMI 1640 culture medium containing 11 mM glucose, 10% fetal calf serum, 100 IU/ml penicillin, 100 μg/ml streptomycin and 50 μg/ml gentamycin. The cells were incubated at 37 °C in 5% CO_2 prior to experiment.

2.2. Cell culture

INS-1 cells were cultured in RPMI 1640 medium supplemented with 50 U/ml penicillin, 50 μg/ml streptomycin, 50 μM 2-mercaptoethanol, 1 mM pyruvic acid, 10 mM HEPES and 10% fetal bovine serum. Cells were used between passages 65 and 100. Cells were grown in the above media containing 3 mM glucose 24 h prior to secretion experiments.

2.3. Amperometry

Six to twelve hours before experiments, serotonin (5-HT, Sigma, St. Louis, MO) was added to the RPMI 1640 culture medium at a final concentration of 1 mM. Glass-encased carbon fiber microelectrodes were pulled from borosilicate glass capillary containing a single carbon fiber of 9 mm diameter (P-55S, Amoco Performance Products, USA). The carbon fibers were sealed in the tip by dipping them in Sylgard (Dow Corning, Kanagawa, Japan) and then cut at a 45° angle. For recordings, the petri dish was placed in a temperature controlled perifusion chamber (32–36 °C), constantly perifused with standard extracellular solution and test substances added as indicated. Standard

☆ Supported by NIH Grant DK35914, The Swedish Research Council (03X-09890, 03XS-12708 and 19X-00034), The European Foundation for the Study of Diabetes, The Swedish Diabetes Association, The Berth von Kantzow's Foundation, The Family Erling-Persson Foundation, The Novo Nordisk Foundation and Funds of Karolinska Institutet.

*Corresponding author. Address: Obesity Research Center, Evans Department of Medicine, Boston Medical Center, EBRC 804, 650 Albany St., Boston, MA 02118, United States. Fax: +1 617 638 7124.
E-mail address: jdeeney@bu.edu (J.T. Deeney).

0014-5793/$32.00
doi:10.1016/j.febslet.2007.07.052

extracellular solution consisted of (in mM): 138 NaCl, 5.6 KCl, 1.2 $MgCl_2$, 2.6 $CaCl_2$, 5 HEPES-NaOH, 3 glucose (pH set to 7.40). Using a micromanipulator (Narishige PE-2, Narishige, Japan), electrodes were placed in close contact with the cell surface and were operated in amperometric mode using an EI-400 potentiostat (Ensman Instrumentation, Bloomington, IN). Records were filtered at 100 Hz (−3 dB value, 8 pole Bessel filter, Frequency Devices, Haverhill, MA) and digitized at 400 Hz (ADC TL-1, Axon Instrument, Foster City, CA). A holding potential of 600 mV, versus a sodium-saturated calomel electrode, was applied to the electrode, which was sufficient to oxidize serotonin [6].

2.4. Serotonin loading

INS-1 cells, grown to a density of 3×10^5 cells per well in 48 well plates, were incubated with 250 μl RPMI 1640 medium per well containing 3 mM glucose and 10 μCi/ml 5-hydroxy-[^{3}H] tryptamine (^{3}H-serotonin) (>100 Ci/mmol, Amersham) for 24 h prior to measurements of secretion. To examine the time course of ^{3}H-serotonin loading, cells were incubated with ^{3}H-serotonin for the indicated times, followed by three washes with Krebs–Ringer bicarbonate buffer. Washed cells were then extracted with phosphate buffered saline; 25 mM NaOH; 0.1% Triton X-100 and aliquots were counted in a scintillation counter to determine CPM of ^{3}H incorporated into the cells.

2.5. Insulin secretion and ^{3}H release

Insulin secretion from INS-1 cells preloaded with serotonin was performed as previously described [18]. INS-1 cells were preincubated for 30 min with Krebs–Ringer buffer containing the same glucose concentration subsequently used to measure basal insulin release. INS-1 cells were then incubated with Krebs–Ringer buffer containing indicated amounts of glucose and KCl for 30 min at 37 °C. The time course of insulin release was measured from individual wells of INS-1 cells stimulated one well at a time with 16.7 mM glucose at 10 s intervals and sampled simultaneously as previously described [18]. Briefly, while incubating at 37 °C the preincubation medium of each well was removed and replaced with buffer containing high glucose in rapid succession. A 10 s/well rate of stimulation was required to resolve the rapid oscillations of insulin release from INS-1 cells.

2.6. Insulin-and ^{3}H release from streptolysin-O permeabilized cells

INS-1 cells preloaded with serotonin were permeabilized with streptolysin-O and Ca^{2+}-stimulated exocytosis was measured as previously described [19].

2.7. Secretion analysis

Samples were analyzed for insulin using radioimmunoassay reagents purchased from Linco Research Inc. (St. Louis, MO). ^{3}H-serotonin release was measured by scintillation counting. Statistical analysis was performed using Student's *t*-test for unpaired data. Oscillations in insulin and ^{3}H-serotonin release were analyzed using the program Optimized Optimal Segments (OOPSEG), a data smoothing program designed to quantitate random measurement error [20].

3. Results and discussion

Serotonin release is routinely used to monitor exocytosis from cells using the amperometric technique [6,7,11]. The technique has been used to measure exoctosis from clonal pancreatic β-cells, dissociated β-cells and whole islets. It has been shown that serotonin loaded into β-cells localizes to the insulin secretory granules and is co-secreted with insulin [10]. Fig. 1 shows typical amperometric results from single ob/ob mouse pancreatic β-cells and clonal insulin-secreting cells (INS-1), which were preloaded with 1 mM serotonin for 6–12 h and stimulated with glucose or KCl. The spikes in the trace represent exocytotic events as the released serotonin was oxidized at the tip of a carbon fiber electrode. Exocytotic activity is shown from INS-1 cells after stimulation with 25 mM KCl (Fig. 1A). In Fig. 1B exocytotic activity from an ob/ob β-cell is shown.

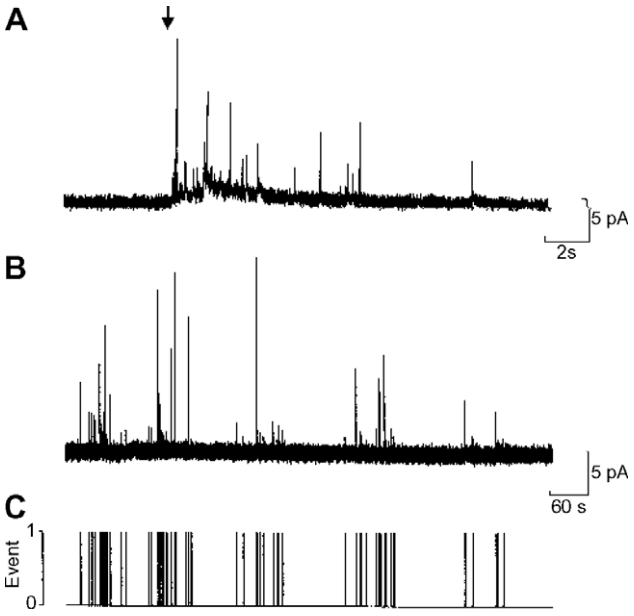

Fig. 1. Amperometric recordings from a single β-cell and INS-1 cell, preloaded with serotonin. Panel A shows a typical example of the secretory response in a single INS-1 cell following stimulation with 25 mM KCl (*arrow*) ($n = 5$). In Panel B, recording from a single ob/ob cell during perfusion with 12 mM glucose is shown. Periods of exocytotic activity occur with a frequency of 60–90 s ($n = 3$). Panel C shows an idealized curve, based on the recording in Panel B. Each line represents an exocytotic event. The recordings were made at 34 °C.

There was very little exocytotic activity at basal, 3 mM glucose, but increasing the glucose concentration to 12 mM caused release of serotonin and thus increased electrode activity. Release of serotonin from the preloaded ob/ob β-cell oscillated with a period of 1–1.5 min, similar to the period of oscillations previously observed in insulin release from INS-1 cells grown in multiwell plates [18].

With these results, we decided to determine how closely ^{3}H-serotonin release reflected the insulin secretory response from populations of INS-1 cells. Fig. 2A depicts the loading pattern of serotonin into INS-1 cells grown for the times indicated in RPMI 1640 media containing ^{3}H-serotonin (10 μCi/ml) and 3 mM glucose. Since most of the label incorporated into the cells was taken up over the first 24 h of incubation, this time was chosen for the cell loading. It has been reported that serotonin concentrations as high as 500 μM have an inhibitory effect on insulin release from perifused rat islets [21]. The high specific activity of the ^{3}H-serotonin used in this study resulted in a final concentration of less than 100 nM, which did not inhibit glucose-induced insulin secretion from INS-1 cells (Fig. 2B).

A comparison of ^{3}H-serotonin release and insulin secretion from serotonin loaded INS-1 cells is shown in Fig. 3. Insulin secretion was stimulated 3- and 10-fold over basal with 16.7 mM glucose and 16.7 mM glucose plus 40 mM KCl, respectively. This is compared to 2- and 4-fold increases in ^{3}H-serotonin release from the same cells under the same conditions. In Fig. 3, ^{3}H-serotonin release at basal 3 mM glucose was arbitrarily set to the same value as insulin release for easy comparison of fold stimulations of release. Although serotonin release appeared to be a good indicator of exocytosis from the INS-1 cells it did not reflect the magnitude of insulin release in

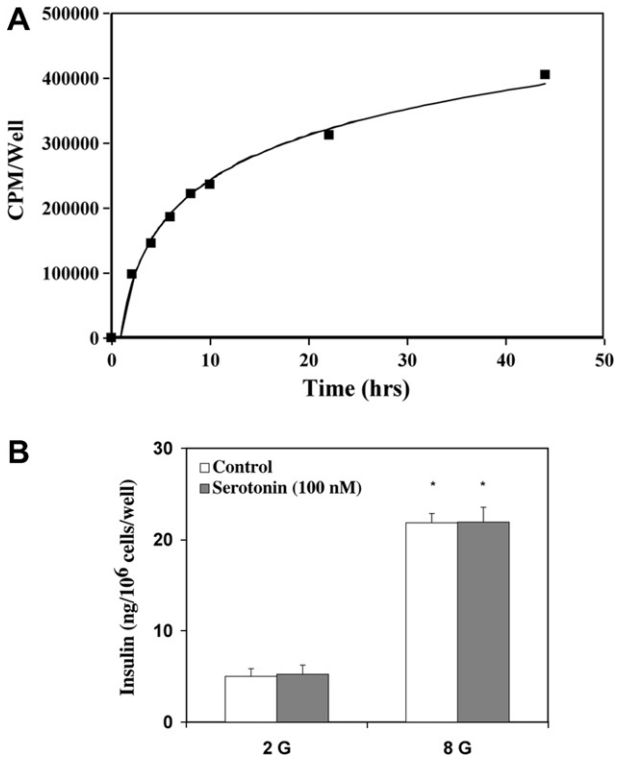

Fig. 2. Time course of ^{3}H-serotonin uptake (A) and effect of serotonin on insulin secretion in INS-1 cells (B). In Panel A INS-1 cells were incubated in RPMI 1640 media containing 3 mM glucose and 10 μCi/ml ^{3}H-serotonin for the times indicated. Cells were washed and extracted as described in methods and values for CPM were determined by scintillation counting. (A) represents a typical experiment repeated three times. Panel B shows 8 mM glucose stimulated insulin secretion 4-fold, compared with the 2 mM glucose basal condition from INS-1 cells preincubated with (shaded bars) or without (white bars) 100 nM serotonin for 24 h. ($n = 2$). Each bar represents the mean of at least five samples and 'n' designates the number of separate experiments. 3-Isobutyl-1-methylxanthine (2.5 μM) was included in the final incubation. $P < 0.001$ as compared to the 2 mM glucose control (∗).

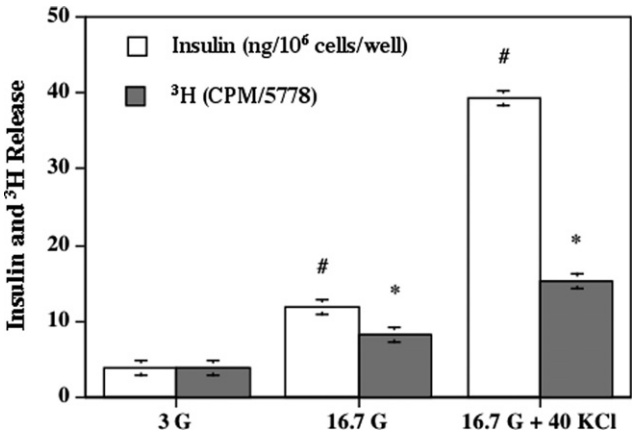

Fig. 3. Comparison of insulin- and ^{3}H-serotonin release from INS-1 cells preloaded with ^{3}H-serotonin. 16.7 mM glucose and 16.7 mM glucose plus 40 mM KCl stimulated insulin secretion from INS-1 cells 3- and 10-fold, respectively compared with the 3 mM glucose basal condition. ^{3}H-serotonin release is shown as shaded bars and increased 2- and 4-fold under the same conditions. In order to facilitate comparison between serotonin and insulin release, ^{3}H-serotonin CPM/well values were divided by 5778 to provide the same numerical value for basal levels of serotonin and insulin release ($n = 3$). Each separate experiment represents the mean of at least three samples and 'n' designates the number of separate experiments. $P < 0.01$ as compared to the 3 mM glucose control for insulin (#) and ^{3}H-serotonin (∗).

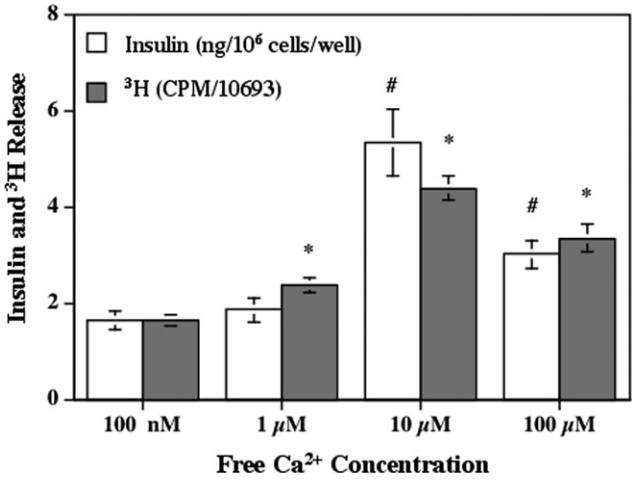

Fig. 4. Comparison of insulin- and ^{3}H-serotonin release from streptolysin-O permeabilized INS-1 cells preloaded with ^{3}H-serotonin. Increasing the free Ca^{2+} from 100 nM to 10 μM resulted in a 3.3-fold increase in insulin release and a 2.6-fold increase in ^{3}H-serotonin release from streptolysin-O permeabilized INS-1 cells. Increasing the free Ca^{2+} to 100 μM resulted in a decrease in both insulin and ^{3}H-serotonin release compared to 10 μM. In order to facilitate comparison between serotonin and insulin release, ^{3}H-serotonin CPM/well values were divided by 10693 to provide the same numerical values of serotonin and insulin release at 100 nM $[Ca^{2+}]_f$ ($n = 3$). Each separate experiment represents the mean of at least three samples and 'n' designates the number of separate experiments. $P < 0.01$ as compared to the 100 nM $[Ca^{2+}]_f$ control for insulin (#) and ^{3}H-serotonin (∗).

the presence of KCl. One possible explanation for this may be variable specific activity of serotonin in the secretory vesicles such that the pool of insulin granules mobilized by depolarization includes granules that are poorly loaded with the ^{3}H-serotonin. Nevertheless, the strong qualitative relationship between serotonin and insulin release makes the use of serotonin, as a marker for insulin release, a valuable tool for comparison of secretion under different conditions.

Insulin- and serotonin-release from streptolysin-O permeabilized INS-1 cells are shown in Fig. 4. In this case, basal release of ^{3}H-serotonin at 100 nM free Ca^{2+} was also arbitrarily set to the same value as insulin release. ^{3}H-serotonin release was increased 2.6-fold by increasing the ambient $Ca^{2+}$from 100 nM to 10 μM. The release of ^{3}H-serotonin from the permeabilized cells was similar to that of insulin.

To determine whether glucose-induced oscillations in insulin release could be monitored by ^{3}H-serotonin release, the time course of insulin release from INS-1 cells grown in multiwell plates was determined. Fig. 5 shows an oscillatory pattern of ^{3}H-serotonin (A) and insulin (C) release from INS-1 cells loaded with ^{3}H-serotonin for 24 h. The time courses for serotonin and insulin release were analyzed using the data smoothing program Optimized Optimal Segments (OOPSEG) in order to mathematically identify oscillations [20]. Oscillations in ^{3}H-serotonin and insulin release, plotted as the derivatives of the smoothed data, are shown in Fig. 5B and D, respectively. The period of oscillations in insulin release from INS-1 cells

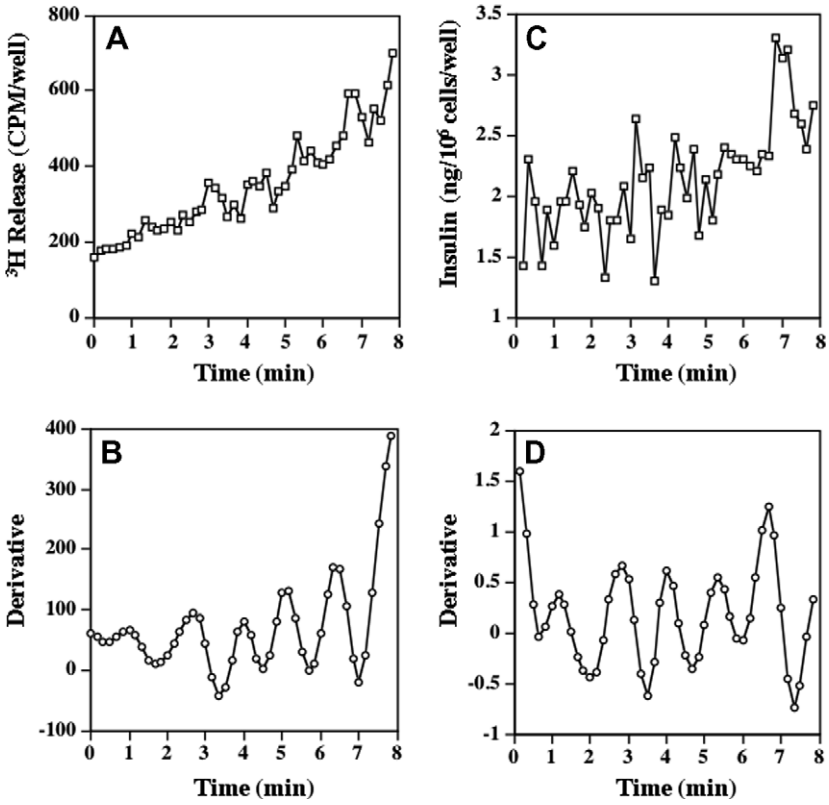

Fig. 5. Oscillations in exocytosis from INS-1 cells grown in multiwell plates as measured by both insulin and ^{3}H-serotonin release. INS-1 cells grown in 48 well plates and loaded with ^{3}H-serotonin were stimulated with 16.7 mM glucose at 10 s intervals and sampled simultaneously in order to obtain a time course of secretion. Panel A shows oscillations in ^{3}H-serotonin released from INS-1 cells grown in multiwell plates while Panel C shows the oscillatory pattern of insulin released from the same cells. Data were smoothed and analyzed using the program OOPSEG. The resulting derivatives of the time courses of serotonin and insulin release are plotted in Panels B and D, respectively. Values for the coefficient of variation is estimated to 8.0% for serotonin release and 11.4% for insulin release, as measured by the OOPSEG program. The figure shows typical results found in three separate experiments.

stimulated with 16.7 mM glucose was 1.5 min, which correlated well with glucose-induced oscillations of ^{3}H-serotonin release from the same cells. Glucose-induced oscillations in insulin secretion from INS-1 cells incubated 24 h with serotonin mimicked oscillations previously measured from INS-1 cells without serotonin pretreatment [18]. The oscillations in exocytosis measured from the population of INS-1 cells grown in the multiwell plate were similar to those measured from single cells using the amperometric technique (cf. Fig. 1).

The similarity between insulin and ^{3}H-serotonin release from preloaded clonal insulin-secreting cells (INS-1) allows for rapid on-line detection of secretion from these cells. This provides a sensitive tool to quickly monitor oscillations in exocytosis, which would facilitate the analysis of coincident oscillations of intracellular metabolites or phosphorylation-dephosphorylation processes. The measurement is dependent on the ability to load cells with serotonin. A second clonal insulin-secreting cell line tested, HIT T-15 cells, incorporated less than 1% of the total ^{3}H counts taken up by INS-1 cells, making the measurements of secretion more difficult in batch incubations and nearly impossible during perifusion. It is interesting to note that measurement of exocytosis from serotonin loaded HIT cells was also not successful using the amperometric technique, suggesting that a component of the serotonin uptake system into these cells is not expressed or functioning. Incorporation of ^{3}H-serotonin was also tested in islets isolated from male Sprague–Dawley rats. Although the incorporation of label was similar to that in INS-1 cells, on a ^{3}H-counts per cell basis, the relatively few cells in the islet compared to the cultured clonal cells in the well made it difficult to follow secretion from single islets using the ^{3}H-serotonin method.

Thus, ^{3}H-serotonin release, as a measure of insulin exocytosis, is an important tool that can be applied to a variety of secretion experiments using clonal insulin-secreting cells. These include release from both intact and permeabilized cells as well as studies of oscillations in exocytosis from INS-1 cells.

Acknowledgements: The authors are indebted to Drs. D.C. Bradley and R.N. Bergman for their kind gift of the OOPSEG program used to analyze oscillations in ^{3}H serotonin and insulin release.

References

[1] Mathieu, C., Waer, M., Casteels, K., Laureys, J. and Bouillon, R. (1995) Prevention of type I diabetes in NOD mice by nonhypercalcemic doses of a new structural analog of 1,25-dihydroxyvitamin D3, KH1060. Endocrinology 136, 866–872.
[2] Stagner, J.I., Samols, E. and Weir, G.C. (1980) Sustained oscillations of insulin, glucagon, and somatostatin from the isolated canine pancreas during exposure to a constant glucose concentration. J. Clin. Invest. 65, 939–942.
[3] Cunningham, B.A., Deeney, J.T., Bliss, C.R., Corkey, B.E. and Tornheim, K. (1996) Glucose-induced oscillatory insulin secretion

in perifused rat pancreatic islets and clonal β-cells (HIT). Am. J. Physiol. 271, E702–E710.
[4] Kennedy, R.T., Huang, L., Atkinson, M.A. and Dush, P. (1993) Amperometric monitoring of chemical secretion from individual pancreatic β-cells. Anal. Biochem. 65, 1882–1887.
[5] Huang, L., Shen, H., Atkinson, M.A. and Kennedy, R.T. (1995) Detection of exocytosis at individual pancreatic beta cells by amperometry at a chemically modified microelectrode. Proc. Natl. Acad. Sci. USA 92, 9608–9612.
[6] Zhou, Z. and Misler, S. (1996) Amperometric detection of quantal secretion from patch-clamped rat pancreatic beta-cells. J. Biol. Chem. 271, 270–277.
[7] Fernandez-Chacon, R. and Alvarez de Toledo, G. (1995) Cytosolic calcium facilitates release of secretory products after exocytotic vesicle fusion. FEBS Lett. 363, 221–225.
[8] Hellman, B., Lernmark, A., Sehlin, J. and Taljedal, I.B. (1972) Transport and storage of 5-hydroxytryptamine in pancreatic β-cells. Biochem. Pharm. 21, 695–706.
[9] Jaim-Etcheverry, G. and Zieher, L.M. (1968) Electron microscopic cytochemistry of 5-hydroxytryptamine (5-HT) in the beta cells of guinea pig endocrine pancreas. Endocrinology 83, 917–923.
[10] Gylfe, E. (1978) Association between 5-hydroxytryptamine release and insulin secretion. J. Endocrinol. 78, 239–248.
[11] Aspinwall, C.A., Brooks, S.A., Kennedy, R.T. and Lakey, J.R.T. (1997) Effects of intravesicular H^+ and extracellular H^+ and Zn^{2+} on insulin secretion in pancreatic beta-cells. J. Biol. Chem. 272, 31308–31314.
[12] Nishizaki, T., Walent, J.H., Kowalchyk, J.A. and Martin, T.F. (1992) A key role for a 145-kDa cytosolic protein in the stimulation of Ca^{2+}-dependent secretion by protein kinase C. J. Biol. Chem. 267, 23972–23981.
[13] Richmond, J.E., Codignola, A., Cooke, I.M. and Sher, E. (1996) Calcium- and barium-dependent exocytosis from the rat insulinoma cell line RINm5F assayed using membrane capacitance measurements and serotonin release. Pflügers Arch. 432, 258–269.
[14] Hellman, B. (1965) Studies in obese-hyperglycemic mice. Ann. N.Y. Acad. Sci. 131, 541–558.
[15] Lacy, P.E. and Kostianovsky, M. (1967) Method for the isolation of intact islets of Langerhans from the rat pancreas. Diabetes 16, 35–39.
[16] Nilsson, T., Arkhammar, P. and Berggren, P.-O. (1987) Extracellular Ca^{2+} induces a rapid increase in cytoplasmic free Ca^{2+} in pancreatic beta-cells. Biochem. Biophys. Res. Commun. 149, 152–158.
[17] Lernmark, A. (1974) The preparation of, and studies on, free cell suspensions from mouse pancreatic islets. Diabetologia 10, 431–438.
[18] Deeney, J.T., Köhler, M., Kubik, K., Brown, G., Schultz, V., Tornheim, K., Corkey, B.E. and Berggren, P.-O. (2001) Glucose-induced metabolic oscillations parallel those of Ca^{2+} and insulin release in clonal insulin-secreting cells; a multiwell approach to oscillatory cell behavior. J. Biol. Chem. 276, 36946–36950.
[19] Deeney, J.T., Gromada, J., Hoy, M., Hervor, L.O., Rhodes, C.J., Prenki, M., Berggren, P.-O. and Corkey, B.E. (2000) Acute stimulation with long-chain Acyl-CoA enhances exocytosis in insulin-secreting cells (HIT T-15 and NMRI β-cells). J. Biol. Chem. 275, 9363–9368.
[20] Bradley, D.C., Steil, G.M. and Bergman, R.N. (1993) Quantitation of measurement error with Optimal Segments: basis for adaptive time course smoothing. Am. J. Physiol. 264, E902–E911.
[21] Zawalich, W.S., Tesz, G.J. and Zawalich, K.C. (2001) Are 5-hydroxytryptamine-preloaded beta-cells an appropriate physiologic model system for establishing that insulin stimulates insulin secretion? J. Biol. Chem. 276, 37120–37123.