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Comparison of Amperometric Methods for Detection of Exocytosis from Single Pancreatic β-Cells of Different Species

Craig A. Aspinwall,[†] Lan Huang,[†] Jonathan R. T. Lakey,[‡] and Robert T. Kennedy*,[†]

Department of Chemistry, University of Florida, Gainesville, Florida 32611-7200, and Department of Surgery, University of Alberta, Edmonton, Alberta, Canada T6G 2N8

Two methods for amperometric detection of exocytosis at single pancreatic β-cells were compared. In the first, direct detection of insulin was accomplished using an insulin-sensitive chemically modified electrode. In the second, 5-hydroxytryptamine (5-HT) that had been allowed to accumulate within the β-cell secretory vesicles was detected with a bare carbon electrode. The goal of the comparison was to determine whether 5-HT secretion was a valid marker of insulin secretion in single β-cells. To aid in this comparison, some experiments involved simultaneous measurement of insulin and 5-HT at cells previously allowed to accumulate 5-HT. Upon application of common insulin secretagogues, current spikes resulting from detection of 5-HT, insulin, or both compounds were obtained indicative of secretion via exocytosis. The mean area of current spikes obtained from simultaneous measurements equaled the sum of the mean area of insulin and 5-HT measured independently. Additionally, analyses of the number of spikes obtained for detection of insulin, 5-HT, or both compounds were similar for several common secretagogues. These data support the hypothesis that accumulated 5-HT is released from insulin containing secretory vesicles, exclusively. In addition, measurement of insulin and 5-HT from β-cells of different species was compared to determine whether a species dependence exists for the two methods compared here. Detection of 5-HT results in a similar number of spikes that are equivalent to insulin in frequency and amplitude in human, porcine, and canine β-cells; however, in mouse and INS-1 β-cells, 5-HT is more readily detected than insulin.

Regulated secretion of hormones and neurotransmitters by exocytosis from individual cells is of critical importance for maintenance of physiological activity in higher organisms. Secretory vesicles typically contain between 0.15 and 10 amol of chemical messenger that is released on the millisecond time scale during exocytosis.[1−4] Amperometry and voltammetry at microelectrodes have been demonstrated to possess the sensitivity and temporal resolution to detect single exocytosis events.[5,6] These techniques have been successfully applied to quantitative measurement of exocytosis from adrenal chromaffin cells,[5−8] PC12 cells,[1] mast cells,[4,9,10] neurons,[11] and melanotrophs,[3,12] providing significant new insight into mechanisms of exocytosis and stimulus−secretion coupling.[4,7,9,13−15] In all of these cases, however, the detected species was relatively easy to oxidize and unmodified carbon-fiber microelectrodes could be used for detection.

We have extended this technique to detection of insulin secretion from single pancreatic β-cells.[2,16,17] For detection of insulin exocytosed from single β-cells, a carbon-fiber microelectrode chemically modified with ruthenium oxide/cyanoruthenate (Ru−O/CN−Ru)[2,16] or ruthenium oxide (RuOx),[17] catalysts that promote oxidation of insulin, is used. This method has allowed the first direct and quantitative measurement of insulin release from single β-cells with high temporal resolution; however, the need for chemically modified microelectrodes to detect insulin has limited application of this technique.

An alternative to direct detection of insulin is measurement of a marker compound, i.e., a substance that is cosecreted with

* Corresponding author: (e-mail) RTKENN@CHEM.UFL.EDU; (phone) 352-392-9839; (fax) 352-392-4582.

[†] University of Florida.

[‡] University of Alberta.

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insulin. It has been suggested that 5-hydroxytryptamine (5-HT) may serve as a suitable marker for insulin secretion in β-cells.[18,19] A particular advantage of 5-HT detection is that it is easily oxidized at unmodified carbon-fiber microelectrodes, negating the need for a chemically modified electrode. While native levels of 5-HT present in β-cells are too low to be detected by amperometry,[2] β-cells will accumulate or "load" 5-HT, reaching vesicle concentrations ~38-fold higher than the extracellular medium.[20,21] Recently, amperometry has been used to measure accumulated 5-HT release from β-cells of a variety of species.[17,22−29] Application of insulin secretagogues results in detection of 5-HT secretion as a series of current spikes that have the characteristics expected of exocytotic release.[19,29] Furthermore, the spikes occur under conditions that are expected to cause insulin secretion. While these studies assume that 5-HT detected is coreleased with insulin exclusively, the data do not exclude release from other vesicle populations within the β-cell. Therefore, further characterization of this method is warranted to validate the use of 5-HT as a marker of insulin secretion.

The primary question that must be addressed is whether 5-HT and insulin are stored and released from the same secretory vesicles exclusively. Ultrastructural histochemistry, fluorescence microscopy, autoradiography, and electron microscopy have provided evidence that accumulated 5-HT is stored in insulin-containing secretory vesicles of the β-cell.[30−33] Thus, exocytosis should result in corelease of 5-HT and insulin. Measurements from whole mouse islets (the β-cell-containing microorgan) have shown that insulin and accumulated 5-HT are both released following glucose stimulation; however, time-resolved measurements of secretion reveal that the ratio of insulin to 5-HT released changes during stimulation.[18] Additionally, the stimulation index (ratio of secretion elicited at 20 mM/3 mM glucose) varies for insulin and 5-HT.[32] Both of these results are unexpected if 5-HT and insulin are coreleased from the same secretory vesicles by exocytosis.

In this work, we compare amperometric detection of insulin and accumulated 5-HT secretion from single pancreatic β-cells of multiple species. The overall goals were the following: (1) to determine whether 5-HT is a valid marker of insulin release, i.e., if the two compounds are coreleased from the same vesicles exclusively, (2) to determine whether the species of β-cell affects detection of insulin and 5-HT, and (3) to determine limitations of using 5-HT release as a marker for insulin secretion.

EXPERIMENTAL SECTION

Chemicals and Reagents. Type XI collagenase, HEPES, 5-hydroxytryptamine, 5-hydroxytryptophan, forskolin, muscarine chloride, and tolbutamide were obtained from Sigma and used without further purification. Ruthenium chloride and potassium hexacyanoruthenate were from Aldrich. All chemicals for islet and cell culture were obtained from Life Technologies. All other chemicals were from Fisher unless noted and were of highest available purity.

Isolation and in Vitro Culture of Mouse β-Cells. Islets were isolated from 20−30-g CD-1 mice following ductal injection with collagenase type XI and dispersed into single cells by shaking in dilute (0.025%) trypsin/EDTA for 10 min at 37° C.[2,34] Cells were cultured at 37° C, 5% CO_2, pH 7.4 in RPMI 1640 containing 10% fetal bovine serum, 100 units/mL penicillin and 100 μg/mL streptomycin and used on days 2−4 after isolation.

Isolation and in Vitro Culture of Canine, Porcine, and Human β-Cells. Pancreatic islets were isolated from canine, porcine, or human pancreas using controlled collagenase (Boehringer Mannheim) perfusion via the duct, automated dissociation, and discontinuous Euro-Ficoll purification using the COBE 2991 blood cell processor as previously described.[35,36] Islets were cultured overnight at room temperature and dispersed into single cells the following day using a previously described procedure.[2,16] Cells were cultured at 37° C, 5% CO_2 in modified CMRL 1066 tissue culture media containing 10% fetal bovine serum, 25 mM HEPES, 100 units/mL penicillin, and 100 μg/mL streptomycin, pH 7.4.

In Vitro Culture of INS-1 Insulin Secreting Tumor Cells. INS-1 cells[37] were provided by Prof. Olof Larsson of the Karolinska Institute. Cells were grown to confluence in tissue culture flasks and then trypsinized with 0.025% trypsin/EDTA. A cell suspension was prepared in modified RPMI 1640 media containing 50 μM 2-mercaptoethanol, 10% fetal bovine serum, 25 mM HEPES, 100 units/mL penicillin, 100 μg/mL streptomycin, 2 mM L-glutamine, and 1 mM pyruvate, pH 7.4. Cells were then plated onto tissue culture plates and maintained at 37° C, 5% CO_2.

Accumulation of 5-HT into β-Cells. For experiments requiring measurement of 5-HT secretion, dispersed β-cells were allowed to incubate for 16 h in tissue culture media containing 0.5 mM 5-HT and 1 mM 5-hydroxytryptophan at 37° C, 5% CO_2 unless stated otherwise. Cells were used for secretion experiments immediately following loading.

Electrode Preparation. Carbon-fiber microelectrodes were constructed as previously described.[16,38,39] Finished electrodes

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consisted of a 9 μm carbon fiber (P-55S, Amoco Performance Products) sealed with epoxy in the tip of a glass pipet. The total electrode diameter at the tip was ~30 μm, and the electrode was polished at a 30° angle using a pipet beveler (Sutter Instruments, BV-10). For detection of insulin and simultaneous detection of insulin with 5-HT, electrodes were chemically modified as previously described to produce a ruthenium oxide/cyanoruthenate (Ru−O/CN−Ru) film.[16] All electrodes were tested for response to the test analytes in a flow injection apparatus. All electrodes used yielded slopes within 10% of 0.5 pA/μM for insulin and 10 pA/μM for 5-HT.

Single-Cell Experiments. Single-cell measurements were performed similar to those described elsewhere.[2,3,6,25] All amperometric experiments were performed on a Zeiss Axiovert 100 inverted microscope. Cells were bathed in pH 7.4 Kreb's Ringer buffer (KRB) containing (in mM), 118 NaCl, 5.4 KCl, 1.2 $MgSO_4$, 2.4 $CaCl_2$, 1.2 KH_2PO_4, 24 $NaHCO_3$, 0.010 forskolin (for canine cells only),[40] and 3.0 D-glucose and maintained at 37° C, 5% CO_2 on the stage of the microscope by a microincubator (Medical Systems, Inc.). Stimulants were prepared in KRB at concentrations indicated in the text. Amperometry at single cells was performed by positioning the sensing tip of the microelectrode 1 μm from the cell using a micromanipulator (Burleigh PCS-250). Stimulant solutions were applied to individual cells by pressure ejection from a micropipet positioned ~30 μm from the cell. To minimize possible effects of electrode fouling from detection of 5-HT, fresh electrodes were used for each cell.

Data Collection. Amperometry was performed using a battery to apply potential to a sodium saturated calomel electrode (SSCE) and a current amplifier (Keithley 428 or Axon AI-403) to measure current at the working electrode. All voltages are versus SSCE. For detection of 5-HT, the potential at the working electrode was 0.65 V, while for detection of insulin, the potential was 0.85 V at the Ru−O/CN−Ru electrode. When the Ru−O/CN−Ru-modified electrode was used, the potential was dropped to 0.40 V between recordings to improve electrode stability as described elsewhere (16). Data were low-pass filtered at 30, 100, or 1000 Hz depending on the experiment. For experiments requiring measurement of spike widths, the higher bandwidth was used. For all cases, the data collection rate was at least 5 times the filter frequency. Data were collected using a personal computer (Gateway 2000 P5-166) via a data acquisition board (Axon DigiData 1200).

Data Analysis. Areas of current spikes were calculated using software provided by Prof. R. M. Wightman (University of North Carolina). For data analysis, spikes were used only if the signal-to-noise ratio was >10 (3 for detection of insulin from mouse and INS-1 β-cells). For measurements of spike area and spike width, only isolated, well-resolved current spikes were used. Statistical means were compared using a two-tailed students *t*-test. Data are presented as the mean ± 1 standard error of the mean (SEM).

RESULTS AND DISCUSSION

Qualitative Analysis of 5-HT as a Marker of Insulin Secretion. To test the hypothesis that insulin and 5-HT are coreleased, we compared results from simultaneous detection of insulin and 5-HT secretion to detection of insulin or 5-HT alone.

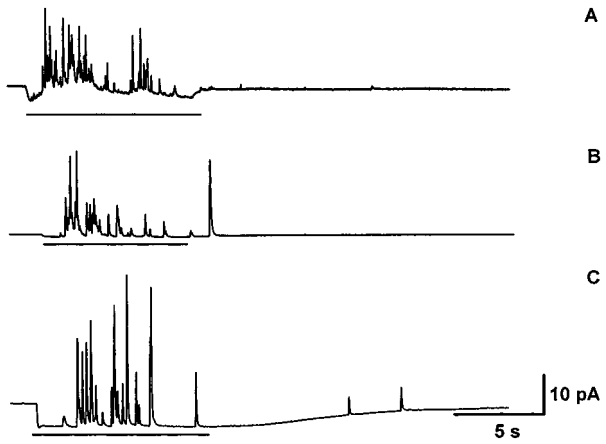

Figure 1. Current recordings of canine β-cells stimulated with 200 μM tolbutamide: (A) detection of insulin using Ru−O/CN−Ru-modified microelectrode from an unloaded cell; (B) detection of accumulated 5-HT using a bare carbon-fiber microelectrode; (C) detection of insulin and 5-HT using a Ru−O/Ru−O electrode. Recordings are from different cells.

Figure 1 shows amperometric recordings obtained from individual canine β-cells upon stimulation with 200 μM tolbutamide made with the following: (a) Ru−O/CN−Ru electrode at "unloaded" cells (i.e., cells not allowed to accumulate 5-HT, therefore measurement of insulin only); (b) carbon-fiber electrode at 5-HT-loaded cells (measurement of 5-HT); and (c) recordings with a Ru−O/CN−Ru electrode at 5-HT-loaded cells. The latter measurement allows both 5-HT and insulin secretion to be detected simultaneously since the Ru−O/CN−Ru electrode detects both insulin and 5-HT. (5-HT sensitivity is the same at both carbon and modified electrodes.) For measurements involving detection and comparison of 5-HT spike areas with simultaneous detection, experiments were performed on cells from the same islet preparation since batch-to-batch variability in the mean spike area was commonly observed for detection of 5-HT (see below). Visual inspection of the data in Figure 1 suggests that spikes resulting from detection of 5-HT, insulin, or both compounds simultaneously are detected with a similar frequency and temporal distribution, which is expected if the two compounds are coreleased from the same vesicles. Additionally, spikes resulting from simultaneous detection of 5-HT and insulin are of larger amplitude than those for insulin or 5-HT alone. Closer analysis of the data as discussed below confirms these initial observations.

Comparison of Mean Spike Areas. Figure 2 compares mean spike areas obtained from detection of insulin, 5-HT, and both compounds simultaneously following stimulation with muscarine, glucose, and tolbutamide, stimulants that elicit exocytosis through different second-messenger pathways. In all cases, the area obtained for simultaneous detection of 5-HT and insulin was equal (within experimental error) to the sum of spike areas resulting from detection of 5-HT or insulin alone. The additive area for simultaneous detection of insulin and 5-HT is expected if 5-HT and insulin are exclusively coreleased from the same vesicles. Release of 5-HT from a second vesicle population, such as the smaller synaptic-like microvesicles (SLMVs) present in the β-cell, would generate detection of some spikes resulting only from oxidation of 5-HT and would result in a lower mean area for

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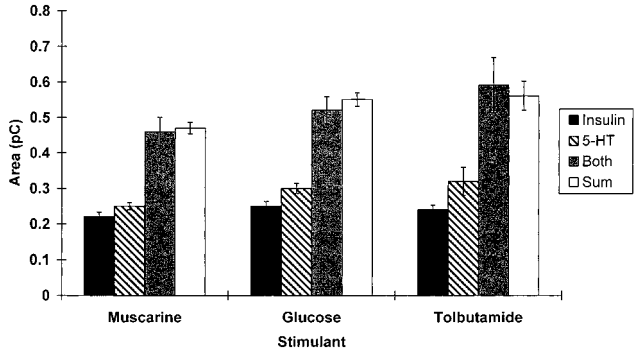

Figure 2. Mean spike areas from stimulation of canine β-cells with, 20 μM muscarine, 17 mM glucose, and 200 μM tolbutamide. Data are presented as mean $\pm$ 1 standard error of the mean. Bars labeled insulin were obtained using Ru−O/CN−Ru-modified microelectrode from an unloaded β-cell. Bars labeled 5-HT are detection of accumulated 5-HT using a bare carbon-fiber microelectrode. Bars labeled both are simultaneous detection of insulin and 5-HT using a Ru−O/CN−Ru electrode. Bars labeled sum are the addition of insulin and 5-HT areas for a given stimulant. In all cases, detection of both compounds simultaneously was statistically significant from detection of insulin or 5-HT alone ($p < 0.0005$).

Table 1. Comparison of Spike Frequency Resulting from Detection of 5-HT, Insulin, or Both Insulin and 5-HT under Different Stimulation Conditions from Canine β-Cells[a]

	analyte		
stimulant	insulin	5-HT	both
tolbutamide	18 ± 1	12 ± 1*	17 ± 2
	$n = 17$	$n = 18$	$n = 27$
muscarine	23 ± 6	19 ± 2	21 ± 3
	$n = 8$	$n = 36$	$n = 12$
glucose	19 ± 4	12 ± 2	19 ± 4
	$n = 13$	$n = 18$	$n = 12$

[a] Data represent the number of current spikes detected per stimulation. Detailed stimulation and analysis conditions given in text. Data presented as mean ± 1 SEM. Asterisk (*) represents statistically significant difference from the number of spikes per stimulation for insulin ($p < 0.01$).

simultaneous detection of insulin and 5-HT than the addition of the individual areas.

Comparison of Frequency of Secretory Activity. Table 1 shows the mean number of current spikes detected per stimulation for insulin, 5-HT, and both compounds simultaneously for tolbutamide, muscarine, and glucose stimulation. In detection of insulin or insulin and 5-HT simultaneously, nearly identical numbers of spikes were observed for all stimulants, suggesting that detection of 5-HT did not result in detection of a additional vesicle population. Detection of 5-HT only typically resulted in a smaller number of spikes than detection of insulin; however, this difference was statistically significant only for tolbutamide stimulation. If 5-HT were released from additional vesicles than those containing insulin, more current spikes should be detected during the simultaneous measurements of both compounds or 5-HT only, not fewer. These results may suggest that not all insulin-containing vesicles are loaded with 5-HT. On the basis of these results combined with the additivity of the mean spike area, we conclude that 5-HT and insulin are coreleased from the same secretory vesicles; however, we cannot rule out the possibility that not all insulin-containing vesicles accumulate 5-HT.

Table 2. Difference in Mean Insulin and 5-HT Spike Area in Four Consecutive Batches of Canine β-Cells[a]

batch no.	insulin area (pC)	5-HT area (pC)
1	0.30 ± 0.03	0.26 ± 0.01[b]
	$n = 49$	$n = 427$
2	0.29 ± 0.02	0.34 ± 0.01
	$n = 145$	$n = 803$
3	0.30 ± 0.02	0.33 ± 0.01
	$n = 230$	$n = 503$
4	0.31 ± 0.02	0.30 ± 0.02[c]
	$n = 84$	$n = 250$

[a] Areas were calculated from well-resolved current spikes with signal-to-noise ratios greater than 10 following stimulation with 200 μM tolbutamide. Data presented as mean ± SEM. [b] Statistically significant difference compared to 5-HT mean spike area from batches 2 and 3 ($p < 0.001$) and from batch 4 ($p < 0.01$). [c] Statistically significant difference compared to 5-HT mean spike area from batch 2 with $p < 0.025$.

Limitations of 5-HT Detection as a Marker for Insulin Secretion. The results presented above indicate that 5-HT can serve as a qualitative marker of secretory activity in canine β-cells; however, it is also important to establish to what extent 5-HT can be used as a quantitative marker of insulin secretion. The area and shape of current spikes are commonly used to elucidate quantitative and kinetic data on single-vesicle release events.[22,24,25] It is therefore of interest to determine whether these characteristics are the same for 5-HT and insulin detection, which would allow experimental factors that affect 5-HT detection to be directly related to insulin secretion.

Table 2 shows the mean spike area for detection of insulin and 5-HT from four consecutive canine β-cell preparations. Small but statistically significant variations in the mean spike area were obtained for 5-HT under identical loading conditions from different preparations while the area of insulin release remained constant. In addition, we observed a dependence of loading time and conditions upon 5-HT mean spike area such that increasing loading time from 2 to 16 h led to a 10-fold increase in 5-HT mean spike area in rodent β-cells. Taken together, the variability of 5-HT spike area from different preparations as well as the dependence on loading conditions demonstrates that great care must be utilized in attempting to relate the amount of 5-HT detected to the amount of insulin released. Reliability can be enhanced if all measurements that are compared are taken on the same preparation of β-cells.

In addition to differences in the amount of 5-HT and insulin released, the kinetics underlying release, reflected in the shape and width of spikes, are quite different for the two compounds. We have previously shown that the width of 5-HT and insulin spikes are differentially affected by changes in the extracellular and intravesicular environment with insulin being affected by H^+ and Zn^{2+} while 5-HT is unaffected.[25] In addition, 5-HT spike widths are increased from 6.4 ± 5.6 ($n = 349$) to 11.9 ± 8.6 ms ($n = 90$) when the extracellular buffer is changed from carbonate to HEPES. Insulin spike widths are unaffected by this change. These results suggest a difference in the mechanism of extrusion from the vesicle for the two compounds such that kinetics observed with 5-HT cannot be extrapolated to insulin.

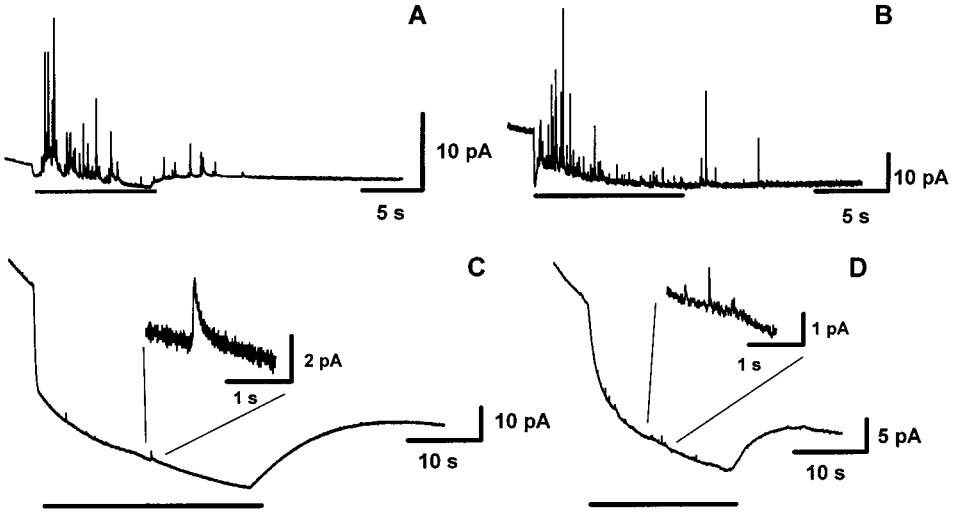

Figure 3. Detection of insulin secretion from single pancreatic β-cell of different species. Detection of insulin with Ru−O/CN−Ru electrode from (A) canine β-cells stimulated with 200 μM tolbutamide, (B) porcine β-cells stimulated with 200 μM tolbutamide, (C) mouse β-cells stimulated with 30 mM K^+, and (D) INS-1 cells stimulated with 30 mM K^+. Insets in (C) and (D) are individual spikes from their respective traces. Bars under traces represent application of stimulant. Drift in the baseline is due to instability of the Ru−O/CN−Ru-modified electrode.

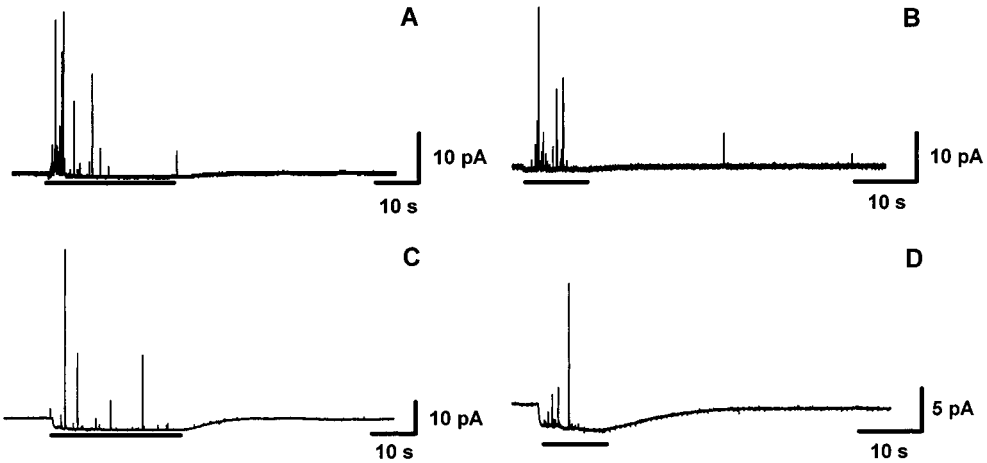

Figure 4. Detection of accumulated 5-HT secretion from a single pancreatic β-cell of different species. Detection of 5-HT with bare carbon electrode from (A) canine β-cells stimulated with 200 μM tolbutamide, (B) porcine β-cells stimulated with 200 μM tolbutamide, (C) mouse β-cells stimulated with 30 mM K^+, and (D) INS-1 cells stimulated with 30 mM K^+. Bars under traces represent application of stimulant.

Detection of Insulin and 5-HT from Other Species of β-Cells. The results above confirm that 5-HT and insulin are coreleased from canine β-cells albeit in different amounts and with different kinetics; however, given the adequate response of the insulin electrode, there is little advantage to detecting accumulated 5-HT rather than insulin in canine β-cells. In contrast, insulin is more difficult to detect in some other species as illustrated in Figure 3, which compares detection of insulin at canine, porcine, mouse, and INS-1 β-cells. These data illustrate that while insulin secretion is readily detected at the canine and porcine β-cells (human cells have a similar responsiveness), insulin detection is more difficult at the rodent cells with spikes being smaller in height and near the noise level. Although these cell types showed a disparity in insulin detection, measurement of 5-HT consistently generated spikes of similar magnitude and frequency at β-cells from all species examined as illustrated in Figure 4. A further comparison is presented in Table 3, which shows that mean area of insulin current spikes and success rate for detection of insulin are significantly lower in mouse and INS-1 cells compared to human, canine, and porcine β-cells while mean area and success rate for 5-HT detection is consistent among the cell types except for a lower area for INS-1 cells.

The difficulty of detecting insulin secretion from the mouse cells may be related to storage of insulin in the vesicles. Insulin is stored within the secretory vesicle as a solid hexamer bound with two Zn^{2+} ions which dissolves and dissociates upon exocytosis.[41] We have shown that an increase in extracellular Zn^{2+} decreases spike area, because more insulin escapes as dissolved complex, which is undetectable, and increases the spike width because the additional Zn^{2+} slows the kinetics of dissolution and dissociation in canine β-cells.[25] Vesicular Zn^{2+} levels are 2.4-fold higher in mouse β-cells than in canine β-cells.[42] Thus, it is likely that the Zn^{2+}−insulin complex released from rodent β-cells

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Table 3. Mean Spike Area and Success Rates for Detection of Insulin and Accumulated 5-HT from Single Canine, Porcine, Human, and Mouse Pancreatic *β*-Cells and INS-1 Cells[a]

	insulin		5-HT	
species	area (pC)	success rate[b] (%)	area (pC)	success rate[b] (%)
canine	0.29 ± 0.01 $n = 571$	38	0.31 ± 0.01 $n = 942$	41
porcine	0.27 ± 0.02 $n = 110$	40	0.26 ± 0.01 $n = 573$	42
human	0.29 ± 0.01 $n = 216$	43	0.29 ± 0.02 $n = 100$	38
mouse	0.20 ± 0.04[c] $n = 12$	9	0.27 ± 0.01 $n = 505$	42
INS-1	0.021 ± 0.003[d] $n = 24$	5	0.15 ± 0.01[e] $n = 528$	41

[a] Areas were calculated from well-resolved current spikes following stimulation with 30 mM K^+ or 200 μM tolbutamide. Data presented as mean ± SEM. [b] Success rate is defined as the percentage of cells that results in detection of at least one current spike following application of stimulant. [c] Statistically significant difference compared to insulin mean spike area from canine, human, and porcine β-cells with $p < 0.01$. [d] Statistically significant difference compared to insulin mean spike area from mouse, canine, human, and porcine β-cells with $p < 0.001$. [e] Statistically significant difference compared to 5-HT mean spike area from mouse, canine, human, and porcine β-cells with $p < 0.001$.

dissociates more slowly due to an excess of Zn^{2+} in the secretory vesicle thereby generating a lower concentration of free insulin monomer at the cell surface. This idea is supported not only by the smaller area of the insulin spikes detected at mouse cells (Table 3) but also by the observation that the mean width at half-height of insulin spikes from canine β-cells is 30 ± 1.3 ms ($n = 153$)[24,25] whereas for the 12 current spikes detected from mouse β-cells the width at half-height was never less than 40 ms. Rat β-cells also have a higher level of Zn^{2+} than canine β-cells,[42] which may make insulin detection at rat β-cells difficult as well.

Detection of Exocytosis in INS-1 Cells. In this work, we also found lower success rates and significantly reduced insulin spike areas from INS-1 cells. It has been reported that the insulin content per INS-1 cell is 20% of that in rat and mouse β-cells even though electron microscopy of INS-1 cells has revealed secretory vesicles of a size similar to the parental rat β-cells.[37] The lower insulin level, combined with the presumed higher Zn^{2+} level because of the rodent origin of the cells, could account for the 10-fold reduction in insulin spike area in INS-1 cells (Table 3). 5-HT detection from INS-1 cells, while quite reliable, did result in spike areas that were significantly smaller than that observed for all other cell types (Table 3). This result is more difficult to explain but could be due to either a greater turnover of vesicles, thus preventing full equilibration of 5-HT, or a difference in vesicle condition, such as higher pH, that lowered the equilibrium content of the 5-HT.

CONCLUSION

The data presented here provide the most conclusive evidence to date that insulin and 5-HT are coreleased exclusively from the same secretory vesicles. Therefore, detection of accumulated 5-HT should serve well as a qualitative marker of vesicle fusion with the caveat that not all insulin vesicles may be loaded with 5-HT. The use of 5-HT as a quantitative marker for spike area or width is problematic because of variability of 5-HT loading from different preparations and differences in the kinetics of release. In species where insulin detection is reliable, such as canine, porcine, and human, direct insulin detection is superior; however, in rodent cells, the poor signal-to-noise ratio for insulin detection makes 5-HT detection the more viable option for amperometric detection of secretion. More sensitive insulin electrodes or methods of decreasing the Zn^{2+} effect may be required to allow better direct insulin detection in rodent cells.

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