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Research Paper

Phasic and tonic modes of depolarization-exocytosis coupling in β-cells of porcine islets of Langerhans

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Abbreviations: [cAMP]i, cytosolic cAMP concentration; PSS, physiological saline solution; [Ca 2+]i, cytosolic [Ca 2+]; QREs, amperometrically measured quantal release events; ΔCm, membrane capacitance change

Key words: β-cell exocytosis, phasic exocytosis, tonic exocytosis, immediately releasable pool, highly calcium sensitive pool

In response to depolarizations that open voltage dependent Ca 2+ channels single porcine β-cells display heterogeneous time courses of exocytosis. Some cells display phasic exocytosis that is triggered by individual or short burst of action potentials typically characteristic of glucose-induced electrical activity or brief voltage clamp depolarization. Other cells, singularly or additionally, display tonic exocytosis that (i) is triggered during prolonged (up to seconds-long) depolarizations to voltages (-30 to -20 mV), and (ii) coincides with rises in global cytosolic [Ca 2+]i >500 nM. We suggest that tonic exocytosis (i) likely results from a recently described pool of granules that is more Ca 2+ sensitive and less co-localized with voltage-sensitive Ca 2+ entry channels than that contributing to phasic exocytosis and (ii) helps tune exocytosis to glucose-induced electrical activity when the latter consists of spike activity followed by intervals of plateau depolarization to nearly -20 mV.

Introduction

Real-time single cell assays of depolarization-exocytosis coupling in pancreatic islet β-cells became available in the early 1990s with precise measurement of membrane capacitance (Cm) tracking membrane surface area, and local electrochemical (amperometric) detection of synchronized or quantal release of preloaded serotonin (by oxidation) or endogenous insulin (by reduction).1-6 From the first of these experiments a “fast-on, fast off” mode of exocytosis was evident as a step increase in membrane capacitance Cm immediately after the conclusion of a brief depolarization or as a barrage of quantal release events during the depolarization.1-5 This phasic exocytosis appears to arise from a small pool of granules, sometimes, called the immediately releasable pool, whose members have a low Ca 2+ affinity and appear to be docked near clusters of high voltage activated Ca 2+ channels.4,6 This exocytosis may account for much of the first phase of glucose induced insulin release from perfused islets.5 However, other records dating back to the same vintage, clearly show that longer depolarizations, especially when applied as a train, produce a “slower-on, slower off” mode of exocytosis monitored as creep-wise increase in Cm and persistence of amperometric discharge lasting up to seconds after the end of the depolarization.5,7-10 This tonic exocytosis, which has received much less attention, may arise from a more highly Ca 2+ sensitive pool of granules docked at some distance from a cluster of Ca 2+ channels. The significance of this component of exocytosis, prominent in human and canine β-cells,8-10 to the time course of insulin release is still unclear.

In our recent survey of stimulus-secretion coupling in porcine β-cells we noted that different cells displayed distinct time course of exocytosis in response to repetitive depolarization. Largely phasic exocytosis is highly synchronized with brief voltage-dependent Ca 2+ entry; largely tonic exocytosis occurs during and after a train of longer depolarizations; and a combination of phasic + tonic exocytosis.11 We have used this preparation to characterize further these modes of exocytosis and consider their differential contribution to exocytosis driven by different patterns of electrical activity.

Results

Heterogeneity of depolarization-induced, Ca 2+-entry-dependent exocytosis from single porcine β-cells. Figure 1 illustrates the key features of the wide heterogeneity of time course of exocytosis displayed by voltage-clamped porcine β-cells. In Ai(i), the three cells depicted all display similar baseline values of Cm (6.3–6.7 pF) as well as similar sized voltage-dependent Ca 2+ currents (ICa(V)) in response to a single depolarization (occurring during the gaps in the Cm trace). In addition, all three cells display similar time courses of increases in cytosolic Ca 2+ concentration,
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[Ca^{2+}], as well as similar total increases in \( C_m \) in response to identical trains of ten 200 ms depolarizations to +10 mV delivered at 1 Hz. However, all three cells display distinct time courses of exocytosis within the train. The cell depicted in the left panel displays “step-wise” increases in \( C_m \) exclusively, with each \( \Delta C_m \) step completed at the end of a depolarization. This likely signifies that granule fusion occurred during the interval of voltage-dependent \( Ca^{2+} \) entry. We shall call this mode of release phasic exocytosis in that it appears to be nearly synchronous with the depolarization. Note, however, that successive depolarizations, though increasing \( [Ca^{2+}] \), to ever higher levels, produced progressively smaller \( C_m \) steps, suggesting rapid depletion of the pool (or diminution of the store) of granules from which this exocytosis was drawn.

In contrast, the cell depicted in the middle panel displays no recognizable \( C_m \) step in response to the first depolarization. However, after the second depolarization it begins to display a slow “creep-wise” increases in \( C_m \) (or \( C_m \) creep), meaning that the change in \( C_m \) largely occurs between depolarizations rather than during these stimulations. This suggests that the exocytosis starts some time into each depolarization and then continues after the depolarization is completed. The rate of \( C_m \) creep accelerates during the subsequent five depolarizations, then decelerates for the remaining of four depolarizations of the train and continued for 2–3 s beyond the end of the train. We shall call this less synchronous mode of discharge tonic exocytosis and suggest that it originates from a pool of granules that does not rapidly deplete and may even be transiently augmented during the depolarization train.

Last, the cell depicted in the right panel displays a combined pattern of \( C_m \) increase: the steps of \( C_m \) seen early in the train seem to deplete, while the creeps of \( C_m \) clearly begin with the third depolarization and increase over the next 3–4 depolarizations. We shall call this phasic + tonic exocytosis. As the distinct patterns of exocytosis occur in neighboring cells with similar diameter, similar \( I_{Ca(v)} \) and similar buildups of \( [Ca^{2+}] \), we shall suggest that these patterns of exocytosis represent intrinsic modes of operation of the exocytotic apparatus.
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Exocytotic heterogeneity in porcine β-cells quantal release as rapidly as 25 ms after onset of depolarization and then ceased to release by 2 s into the sustained depolarization. However, the cell in the right panel, labeled tonic exocytosis, commenced its quantal release at the earliest only after 200 ms of onset of depolarization, while continuing it, though at decreasing rate, for the entire 3.5 s of depolarization and then for ~1.5 s thereafter. This comparison suggests that in phasic exocytosis the entering Ca\textsuperscript{2+} sets off highly localized release from a small pool of immediately releasable granules likely docked near Ca\textsuperscript{2+} entry channels, while in tonic exocytosis the entering Ca\textsuperscript{2+} must accumulate and diffuse to reach and discharge a larger more dispersed pool of granules at some distance from the Ca\textsuperscript{2+} entry channels.

In total, of the 23 cells examined in one of the recording conditions shown here, six exhibited phasic exocytosis exclusively, seven tonic exocytosis exclusively, while ten showed combined phasic + tonic exocytosis pattern, in which it was possible to extinguish the phasic component by repeating the depolarization train within 20–30 s of the prior train.

Minimal conditions for phasic exocytosis (nearly synchronous with duration of depolarization) vs. tonic exocytosis (slowerto-start and slow-to-stop). Figure 2 illustrates two sets of experiments designed to quantify the minimal Ca\textsuperscript{2+} entry needed to provoke detectable quantal release and determine whether single action potentials seen in porcine β-cells are capable of providing this.
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First, as shown in (A), robustly secreting porcine β-cells were tested with single, brief (20–35 ms) depolarizations applied in random sequence at 20–30 s intervals. Note that the amplitude of resultant C_m steps was clearly voltage-dependent and closely paralleled Q_{Ca}. Little if any increase in C_m was seen at voltages negative to -25 mV; however the amplitude of C_m steps increased sharply with increasing depolarization, peaking at +10 mV, where Q_{Ca} is maximal. Thereafter, the amplitude of C_m steps sharply decreased with larger depolarization. While 15 ms-long depolarizations to -20 mV evoked no detectable increase in C_m over 10 trials, 15 ms-long depolarizations to -10 mV, which provoked 2-fold greater increases in Ca^{2+} entry, evoked highly reproducible steps of C_m averaging 20 fF, or roughly equivalent to the fusion of 7–10 insulin granules. Increasing the duration of depolarization to 30 ms resulted in readily detectable C_m steps even at -20 mV, with 7/10 such depolarization trials producing C_m steps at least 5 fF in amplitude. In a set of six β-cells, in which we had previously recorded action potentials evoked by 12 mM glucose under current-clamp conditions, we noted that at least 70% (108/168) of the action potentials spent at least 30 ms at potentials equal or positive to -20 mV (i.e., -20 to +10 mV). This suggested that in robustly secreting β-cells the majority of single action potentials might be capable of evoking detectable quantal release.

Second, to test this hypothesis more directly, in several β-cells we investigated the effectiveness of various simulated action potential (AP) waveforms, applied as complex voltage-clamp sequences, in evoking increases in C_m. Examination of effects of changes in action potential shape on calcium currents and transmitter release has proven a useful tool for examining synaptic transfer curves across central synapses and depolarization-exocytosis coupling at peripheral nerve terminals. In B, the top row presents traces (1) and (2), representing the extremes of shape of glucose-provoked APs recorded from porcine β-cells; the second row presents a range of voltage-clamp simulated APs, including those resembling the sample native APs, here labeled (1') and (2'); the third row presents the corresponding Ca^{2+} currents (I_{Ca(v)}) the simulated APs evoke. Of note is that I_{Ca(v)}s occur largely during the decay phase of the action potential and that for a given peak depolarizing voltage, the longer the decay phase, the longer the I_{Ca(v)} duration and the more intense the total Ca entry, Q_{Ca}. Also, simulated APs of different waveforms (e.g., 1' and 2') can evoke I_{Ca(v)}s of quite different shapes but of very similar total Q_{Ca}s. The graph at the bottom of B presents a summary of C_m vs. Q_{Ca} compiled from records of five robustly exocytosing cells subjected to the range of the simulated APs shown. In four of the five cells, simulations of the sample APs were competent in evoking reliably measured increases in C_m.

Figure 3 illustrates a set of experiments designed to test the range of global [Ca^{2+}], needed to evoke tonic exocytosis and to compare this with the range of global [Ca^{2+}], achieved during plateau depolarizations. In A, repetitive 50 or 100 ms depolarizations to -15 mV were applied to Fura-2AM-preloaded cells displaying both phasic and tonic components of exocytosis. Tonic exocytosis, characterized by the “creep-wise” increase in C_m, though only occasionally seen late into a train of 50 ms pulses, was routinely evident by the third or fourth pulse within a train of 100 ms pulses. The appearance of tonic exocytosis coincided with rises in [Ca^{2+}], to levels exceeding an estimated value >500 nM, in spite of little evidence of C_m steps. However, as is apparent in B, the rate of C_m creep (creep dC_m/dt), measured as the slope of the C_m curve, increased steeply as a supralinear function of [Ca^{2+}] over the range 500–1,000 nM. As shown immediately below, this correlates well with the range of [Ca^{2+}] measured during the intermittent plateau depolarizations that develop in many porcine β-cells during prolonged glucose-induced depolarization.

Evidence for plateau depolarization—type electrical activity to support sustained Ca entry and tonic exocytosis. In current-clamped, Fura-2 loaded porcine β-cells elevation of [glucose]_o to 10–12 mM results, within minutes, in the appearance of i) single action potentials that give rise to nearly simultaneous spikes of
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Increases in [Ca^{2+}] and (ii) clusters of APs that give rise to more prolonged bursts increases in [Ca^{2+}]. This coupling of action potential and [Ca^{2+}], transient activity may last for the duration of the cells’ exposure to elevated [glucose]o (see Fig. 1D in companion paper and Fig. 4A here). However, with exposures to elevated [glucose]o for greater than 8–10 min it is not unusual for intermittent plateau depolarizations (PD), lasting from 10 to 55 s at a V_{m} between -25 and -15 mV, to arise after a train of action potentials. These PDs bring [Ca^{2+}] to values between 500 and 1,000 nM (see Fig. 4B). We call these sustained [Ca^{2+}] transients giant plateaus. PDs end in abrupt repolarizations of V_{m} to -55 to -60 mV lasting 10 to 15 s and accompanied by recovery of [Ca^{2+}], to a baseline of 100 to 200 nM. Thereafter V_{m} depolarizes to between -45 and -40 mV and the cell resumes its firing of irregular action potentials accompanied by spike or burst-like [Ca^{2+}] transients.

We observed these PD/giant plateau combinations in 9/12 current-clamped, Fura-2 loaded porcine β-cells whose responses to elevated [glucose]o were monitored for longer than 9 min. In these cells, in the interval between 7–15 min in [glucose]o = 12 mM, the fraction of time a cell spent in the PD/giant plateau mode was 3–12%.

Increases in [cAMP] enhance phasic exocytosis and reveal tonic exocytosis. In our companion study,11 confirming prior studies of β-cells in other species15,16 we showed that augmenting [cAMP], either by applying a membrane permeant derivative or by stimulating adenylate cyclase, via binding a G-protein coupled receptor for glucagon or glucagon-like intestinal peptide 1 (GLP-1), increased single cell insulin granule exocytosis, measured as ΔC_{m}. Often either maneuver produced little or no enhancement of depolarization-induced calcium entry. In Figure 5 we have re-examined this finding in porcine β-cells in the light of our dissection of phasic from tonic modes of depolarization-induced exocytosis. Panel A presents data from a single cell demonstrating that raising [cAMP] by exposure of cells previously untreated with forskolin to the membrane permeable analog 8-CPT-cAMP, enhances phasic exocytosis (C_{m} steps immediately following the initial 2–3 depolarizations in a train), as well as recruits tonic exocytosis (C_{m} creeps after the remaining depolarizations in the train) not at all appreciated under control conditions. Summarizing data from five cells, Panel B demonstrates that, on average, a 6 min incubation of forskolin-naïve cells with 8-CPT-cAMP produces on average a 3-fold increase phasic exocytosis (as defined above) as well as a near 20-fold increase tonic exocytosis (as defined above), as compared with control conditions.

Discussion

Many porcine β-cells display a “fast on-fast off” pattern of exocytosis that is synchronous with depolarization. This phasic exocytosis closely resembles the major mode of secretion reported in varying detail in rodent β-cells, from the inception of single cell assays of exocytosis and thought to contribute to the bulk of first phase glucose-induced insulin secretion from these cells.1-6 Here we demonstrate that porcine β-cells display phasic exocytosis in response to single brief depolarizations to clamping potentials positive to -20 mV; it consists of a burst of quantal release events (QREs) during the effective depolarization and step increase in C_{m} immediately after cessation of the depolarization, with the intensity of the QRE burst and the magnitude of the step increase in C_{m}, increasing with increasing Ca^{2+} entry. Phasic exocytosis is also seen during a train of depolarizations; however, under these conditions both the burst of QREs during, and the step increase in C_{m} following, each interval of depolarization decreases with pulse repetition, even as the average cytosolic [Ca^{2+}] progressively increases. The latter suggests that the pool of granules contributing to phasic exocytosis, though almost immediately useable, is of limited capacity and hence rapidly depleting. Application of cell attached patch recording to mouse β-cells has shown that the opening of 1–2 Ca^{2+} channels results, within a few milliseconds, in a small (1.5–2.5 pF) step increase in C_{m} consistent with the fusion of a 250 nm diameter insulin granule with the plasma membrane.4,5 In the latter cells, the small pool of ~50–100 insulin granules contributing to phasic exocytosis is also released when the submembrane [Ca^{2+}] is raised to the level of >10 μM by vigorous flash photolysis of caged Ca^{2+} compound previously injected into the cytoplasm.6 This “immediately releasable pool” (IRP) of granules is likely localized very near the internal mouths of a small group of Ca^{2+} channels, since at larger distances from the channels the amplitude of the Ca^{2+} micro-domain would rapidly fall below the threshold concentration of >10 μM required for triggering release of granules comprising this pool.

However, the majority of porcine β-cells also display, either alone or in combination with the phasic exocytosis, a “slow on-slow off” pattern of exocytosis, which we call tonic exocytosis. The latter consists of the steady appearance of quantal release events,
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Asynchronous with depolarization and accompanied by a progressive, “creeping” increase in C_m. Both the quantal release events and the increase in C_m begin as late as hundreds of ms into, and continue for up to several seconds after cessation of, a prolonged depolarization, to voltages positive to -30 mV. Tonic exocytosis first appears after total Ca^{2+} entry increases global [Ca^{2+}]_i to > 500–600 nM, as measured by slowly responding Ca^{2+} indicator dyes such as Fura compounds. At higher [Ca^{2+}]_i, the rate of this mode of exocytosis rises steeply. We assume that the average [Ca^{2+}]_i, measured by imaging the cytoplasm globally, applies to this mode of exocytosis rises steeply. We assume that the average [Ca^{2+}]_i, measured by imaging the cytoplasm globally, applies to the region immediately beneath the plasma membrane because in similarly sized adrenal chromaffin cells admitting similar Ca^{2+} currents, confocal microscopy reveals that [Ca^{2+}]_i, reaches a uniform global level after ~200 ms depolarization to voltages (~10 to 0 mV) where Ca^{2+} current is near maximal.18 This tonic mode of exocytosis was clearly present in the C_m responses to trains of depolarization recorded in early experiments with rodent β-cells, and was massively enhanced when external Ca^{2+} was replaced with Sr^{2+} or Ba^{2+}.2,7 However, tonic exocytosis has merited little comment or discussion and its physiological significance has only been considered in recent literature on human β-cells.8–10

In contrast to phasic exocytosis, if the insulin granules contributing to tonic exocytosis constitute a discrete pool, it is likely that its member granules are located far from Ca^{2+} channels and have a much higher Ca^{2+} sensitivity. Ca^{2+} entering the cell via Ca^{2+} channels would need to (i) diffuse a longer distance, as well as (ii) avoid capture by both fixed and diffusible cytosolic Ca^{2+} buffers, to reach the release trigger sites associated with these granules. As initial Ca^{2+} entry would need to saturate these cytosolic buffers before sufficient Ca^{2+} were able to bind to granule trigger sites, onset of tonic release would be delayed by as long as hundreds of milliseconds after the onset of depolarization, as compared with phasic release, where the trigger is the high intensity burst. Recently, a novel component of the so-called readily releasable pool of granules has been described in rodent β-cells and β-cell-derived lines; it is discharged by less exhaustive flash photolysis of caged Ca^{2+} compounds raising [Ca^{2+}]_i to between >0.5 and <5 μM and is called a highly Ca sensitive pool (HCSP).6 This precisely the range of [Ca^{2+}]_i that (i) induces similar slow rates of continuous exocytosis in β-cells either dialyzed via whole cell patching with pipettes containing buffered Ca^{2+} solutions2–7 or permeabilized to extracellular Ca^{2+} by the channel forming neurotoxin α-latrotoxin9 and (ii) induces the slow rates of insulin secretion displayed by digitonin-permeabilized β-cells.19 The size of the HCSP is greatly augmented by pre-treatment of the cell with pharmacological stimulators of protein kinases A and C or by raising the ambient concentration of glucose but remains largely intact after a bout of repetitive stimulation that depletes the IRP.6 In preliminary experiments with single human β-cells displaying both the tonic pattern of exocytosis we were able to identify a corresponding HCSP.10

In the experiments we report here, where β-cells were exposed to enhancement of PKA (by forskolin) and near stimulatory levels of glucose, it is likely that the HCSP was particularly enhanced in prominence. In fact, Figure 5 demonstrates that addition of a membrane permeable analog of cAMP dramatically recruits tonic exocytosis in response to repetitive depolarization, under conditions where previously it was hardly apparent.

Figure 5 presents a summary schematic for the relative contributions of phasic and tonic modes of exocytosis (and their presumed sources, the IRP and HCSP respectively) to the release of insulin granules evoked both during and after depolarizations of increasing duration that initially raise local [Ca^{2+}]_i near the mouths of a cluster (or “hot spot”) of voltage dependent Ca^{2+} channels to >20 μM. As the duration of the depolarization increases, continuing Ca^{2+} entry produces an expanding domain of submembrane increase in [Ca^{2+}]_i. Within several ms of the start of Ca^{2+} entry, the diffusing Ca^{2+} is able to trigger exocytosis of granules of the IRP that are docked within nanometers of the Ca^{2+} channel “hot spot” and bind Ca^{2+} with a relatively low affinity. This produces the “step” increase in C_m seen after depolarization, where the step size is a function of duration of depolarization. When Ca^{2+} channels continue to remain open, within 10 s of ms the continually entering Ca^{2+} diffuses far enough so that a much lower concentration reaches, and hence triggers exocytosis from, granules of the HCSP, which, though docked far from Ca^{2+} channel “hot spot”, bind Ca^{2+} with a relatively higher affinity. On cessation of Ca^{2+} current, the peak of [Ca^{2+}]_i near the...
Ca\(^{2+}\) channel “hot spot” rapidly collapses to levels below where it is able to trigger exocytosis from IRP. However, the local [Ca\(^{2+}\)]\(_i\) at some distance from the hot spot is likely to remain elevated and exceed the threshold for triggering release from the HCSP for up to seconds thereafter (i.e., until intracellular and plasma membrane Ca\(^{2+}\) transporters re-establish Ca\(^{2+}\) homeostasis). This produces the slower, more continuous “creeping” rise in \(C_m\) seen after the step increase in \(C_m\) under conditions of prolonged depolarization. Given this model it is not unreasonable to predict that at prolonged over several seconds, might still contribute enough intracellular stores, should directly or indirectly increase the sizes of the IRP as well HCSP, and hence further enhance phasic as well as tonic exocytosis. Second, unfortunately our data does not allow us to directly comment on either (i) the timing of exocytotic release of insulin in either the phasic or tonic release mode (i.e., whether insulin is released as rapidly as C-peptide\(^{22}\) or (ii) the precise timing of granule membrane endocytosis (e.g., rapid kiss-and-run mode of fusion/fission\(^{23}\)).

The joint contribution of both tonic and phasic exocytosis to quantal release during sustained activity is a well appreciated phenomenon in excitable cells. First observed at the neuromuscular junction as (i) increased miniature endplate potential activity...
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interspersed between individually evoked endplate potentials\(^{24,25} \) and (ii) as sustained miniature endplate potential frequency during nerve terminal depolarizations that do not trigger action potentials.\(^{26} \) It has more recently been seen in the outputs of rapidly firing central inhibitory interneurons\(^{27} \) and as well as the adrenal chromaffin cells driven by a train of action potentials.\(^{28} \) Also, time courses of increases in \( C_{m} \), very similar to what we define as phasic and tonic, have previously been reported from peptidergic neurohypophyseal terminals and adrenal chromaffin cells, though these results were interpreted in a different fashion.\(^{29,30} \) It is worth noting that the adrenal chromaffin cell also has clearly definable IRP and HCSP.\(^{31} \) In the chromaffin cell, synaptotagmin-1 (syt-1), the canonical synaptotagmin isoform identified as the \( \text{Ca}^{2+} \) sensor for fast synchronous exocytosis forebrain neurons, appears to be the sensor for phasic exocytosis, while an alternative isoform, syt-7, for fast synchronous exocytosis in \( \beta \)-cells, the situation is less clear: while the role of syt-1 is unclear, there is new evidence of impaired insulin secretion (second phase \(<40 \mu \text{M} \) was added to porcine \( \beta \)-cells,12 and are direct applications of those used for the study of human and canine \( \beta \)-cells. \(^{9,10} \) In experiments where exocytosis was directly measured, forskolin (10 \( \mu \text{M} \)) was added to the appropriately modified physiological saline solution (PSS), to increase the efficiency of coupling of depolarization to secretion without interfering with electrochemical detection of exocytotic release of insulin granule contents.\(^{2,12} \) To record whole-cell currents simultaneously with membrane capacitance, patch pipettes were filled with high \( \text{Ca}^{2+} \) internal solution (\( \text{Ca}^{2+} \)-IS) containing, in mM: 63.7 \( \text{CsCl} \); 28.35 \( \text{CaSO}_{4} \); 47.2 sucrose; 11.8 \( \text{NaCl} \); 1 \( \text{MgCl}_{2} \); 0.5 mM EGTA and 20 HEPES titrated to pH 7.35 with \( \text{NaOH} \). In experiments where glucose-induced electrical activity was recorded, an alternative high K\(^{+} \) internal solution (K\(^{+} \)-IS), in which K\(^{+} \) replaced \( 	ext{Ca}^{2+} \) on a mole-for-mole basis, was used. As discussed in the companion paper,\(^{11} \) the cells chosen for study by single cell electrical, electrochemical and calcium imaging techniques were the largest single cells seen (\( \approx 12 \mu \text{m} \) in diameter and \( >5.5 \mu \text{m} \) in baseline cell capacitance), criteria previously used to identify \( \beta \)-cells in other species and supported by our limited recordings from smaller cells, which displayed features more characteristic of \( \alpha \)-cells in other mammalian species. Current-clamp recording was begun after the pipette-to-cytoplasm access resistance (\( R_{p} \)) fell to \(<40 \) M\( \Omega \) while voltage clamp recording was begun after \( R_{p} \) fell to \(<30–25 \) M\( \Omega \), with the cell initially stepped from a holding potential of \(-70 \) mV. Whole cell currents were leak subtracted using a standard p/4 protocol. Quantal release events (QREs) were monitored by amperometry using a carbon fiber electrode (tip potential \( = +650 \) mV) positioned at the surface of cells that were previously loaded with serotonin (5-HT) and 5-hydroxytryptophan, at incubation concentrations of 0.5 mM each, and then bathed in a PSS containing 4 mM glucose and 10 \( \mu \text{M} \) forskolin that was maintained at 32°C. Membrane capacitance changes (\( \Delta C_{m} \)) following cell depolarization were estimated using an \( \text{EPIC} \)-9 patch clamp amplifier (Heka, Electronic, Lambrecht, Germany) and software-based, dual frequency lock-in detector (LID) developed as a set of extensions (XOP modules) of the numerical/graphics package Igor (Wavemetrics, Inc., Oregon, USA) to (i) apply dual-frequency, small signal voltage excitation (10 mV peak-to-peak at 400 and 800 Hz) to the cell held at a DC potential of \(-70 \) mV and (ii) subsequently process the current response.

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References

1. Gillis KD, Misler S. Single cell assay of exocytosis from pancreatic islet \( \beta \)-cells. Pfluegers Arch 1992; 420:121-3.
2. Ammalu C, Eliasson L, Boekvit K, Larson O, Ashcroft FM and Rorsman P. Exocytosis elicited by action potentials and voltage-clamp calcium currents in individual mouse pancreatic beta-cells. J Physiol 1993; 472:665-88.
3. Zhou Z and Misler S. Amperometric detection of quantal secretion from patch-clamped rat pancreatic \( \beta \)-cells. J Biol Chem 1996; 271:270-7.
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4. Huang L, Shen H, Atkinson MA, Kennedy RT. Detection of exocytosis at individual pancreatic β-cells by amperometry at a chemically modified microelectrode. Proc Natl Acad Sci USA 1995; 92:9608-12.

5. Burg S, Ma X, Eliasson L, Galvanovskis J, Gopel SO, Obermuller S, et al. Fast exocytosis with few calcium channels in insulin-secreting mouse pancreatic B cells. Biophys J 2001; 81:3508-23.

6. Barg S, Eliasson L, Renstrom E, Rorsman P. A subset of 50 secretory granules in close contact with L-type Ca2+ channels accounts for first-phase insulin secretion in mouse beta-cells. Diabetes 2002; 51:74-82.

7. Yang Y, Gillis KD. A highly Ca2+-sensitive pool of granules is regulated by glucose and protein kinases in insulin-secreting INS-1 cells. J Gen Physiol 2004; 124:641-51.

8. Barnett DW, MISLER S. Coupling of exocytosis to depolarization in rat pancreatic islet beta-cells: effects of calcium, strontium and barium containing extracellular solutions. Pflugers Arch 1995; 430:593-5.

9. MISLER S, DICKEY A, BARNETT DW. Maintenance of stimulus-secretion coupling and single beta-cell function in cryopreserved-thawed human islets of Langerhans. Pflugers Arch 2005; 450:395-404.

10. Silva AM, Liu-Gentry J, Dickey AS, Barnett DW and MISLER S. α-Latrotoxin increases spontaneous and depolarization-evoked exocytosis from pancreatic islet β-cells. J Physiol 2005; 565:783-99.

11. MISLER S, BARNETT DW, Gillis KD. Update on Electrophysiology of Stimulus-Secretion Coupling in Human β Cells. In “Retrospectives on Prospectives in Diabetes”, Robertson RP, ed 2006.

12. Silva AM, Dickey AS, Barnett DW, MISLER S. Ion channels underlying stimulus-exocytosis coupling and its cell-to-cell heterogeneity in β-cells of porcine islets of Langerhans (companion MS submitted to Channels) 2009.

13. Barnett DW, Pressel DM, Chern HT, Scharp DW, MISLER S. cAMP-enhancing agents “permit” stimulus-secretion coupling in canine pancreatic islet beta-cells. J Membrane Biol 1994; 138:113-20.

14. Grynkiewicz G, Poenie M, Tsien RY. A new generation of Ca2+ indicators with greatly improved fluorescence properties. J Biol Chem 1985; 260:3440-50.

15. Borst JGG, Sakmann B. Effect of changes in action potential shape on calcium currents and transmitter release in the calyx-type synapse of the rat auditory brainstem. Phil Trans Royal Soc Lond. B 1999; 354:347-55.

16. Ammala C, Ashcroft FM, Rorsman P. Calcium independent potentiation of insulin release by cyclic AMP in single β-cells. Nature 1993; 363:356-8.

17. Gillis KD, MISLER S. Enhancers of cystolic cAMP augment depolarization-induced exocytosis from pancreatic β-cells: evidence for effects distal to Ca2+ entry. Pflugers Arch 1993; 424:195-7.

18. Gromada J, Bokvist K, Ding WG, Holst JJ, Nielsen JH, Rorsman P. Glucagon-like peptide 7–37 amide stimulated exocytosis in human pancreatic beta-cells by both proximal and distal regulatory steps in stimulus-secretion coupling. Diabetes 1998; 47:57-65.

19. Augustine GJ, Néher E. Calcium requirements for secretion in bovine chromaffin cells. J Physiol 1992; 450:247-71.

20. Jones PM, Persaud SJ, Howell SL. Calcium-induced insulin secretion from electrically permeabilized islets. Biochem J 1992; 285:973-8.

21. Zhang Q, Galvanovskis J, Abdulkader F, Partridge CJ, Gopel SO, Eliasson L, Rorsman P. Cell coupling of mouse β-cells measured in intact islets of Langerhans. Phil Trans Royal Soc A 2008; 366:3503-23.

22. Michael DJ, Rizell RA, Haataja L, Chow RH. Pancreatic β-cells secrete insulin in fast and slow release forms. Diabetes 2006; 55:600-7.

23. Hanna ST, Pigeau GM, Galvanovskis J, Clark A, Rorsman P, MacDonald PE. Kiss-and-run exocytosis and fusion pores of secretory vesicles of human β-cells. Pflugers Arch 2008; (in press, on line publication).

24. Liley AW. The quantal components of the mammalian end-plate potential. J Physiol 1956; 135:571-87.

25. Hurlbur WP, Longenecker HB, Mauro A. Effects of calcium and magnesium on the frequency of miniature end-plate potentials during prolonged tetanization. J Physiol 1971; 219:17-38.

26. Liley AW. The effects of presynaptic polarization on the spontaneous activity at the mammalian neuromuscular junction. J Physiol 1956; 134:427-43.

27. Zhou Z, MISLER S. Action potential-induced quantal secretion of catecholamines from rat adrenal chromaffin cells. J Biol Chem 1995; 270:3408-505.

28. Heft S, Jonas P. Asynchronous GABA release generates long-lasting inhibition at a hippocampal interneuron-principal neuron synapse. Nature Neuroscience 2005; 8:1319-28.

29. Seward EP, Nowycky MC. Exocytosis in peptideergic nerve terminal exhibits two Ca-sensitive phases during pulsatile calcium entry. J Neurosci 1995; 15:3390-9.

30. Seward EM, Nowycky MC. Kinetics of stimulus-coupled secretion in dialyzed bovine chromaffin cells in response to trains of depolarizing pulses. J Neurosci 1996; 16:553-62.

31. Yang Y, Udayasankar S, Dunning J, Chen P, Gillis KD. A highly Ca2+ sensitive pool of vesicles is regulated by protein kinase C in adrenal chromaffin cells. Proc Natl Acad Sci USA 2002; 99:17060-5.