Regulated Exocytosis and Kiss-and-Run of Synaptic-Like Microvesicles in INS-1 and Primary Rat β-Cells

Patrick E. MacDonald, Stefanie Obermüller, Jenny Vikman, Juris Galvanovskis, Patrik Rorsman, and Lena Eliasson

We have applied cell-attached capacitance measurements to investigate whether synaptic-like microvesicles (SLMVs) undergo regulated exocytosis in insulinoma and primary pancreatic β-cells. SLMV and large dense-core vesicle (LDCV) exocytosis was increased 1.6- and 2.4-fold upon stimulation with 10 mmol/l glucose in INS-1 cells. Exocytosis of both types of vesicles was coupled to Ca\(^{2+}\) entry through L-type channels. Thirty percent of SLMV exocytosis in INS-1 and rat β-cells was associated with transient capacitance increases consistent with kiss-and-run. Elevation of intracellular cAMP (5 μmol/l forskolin) increased SLMV exocytosis 1.6-fold and lengthened the duration of kiss-and-run events in rat β-cells. Experiments using isolated inside-out patches of INS-1 cells revealed that the readily releasable pool (RRP) of SLMVs preferentially undergoes kiss-and-run exocytosis (67%), is proportionally larger than the LDCV RRP, and is depleted more quickly upon Ca\(^{2+}\) stimulation. We conclude that SLMVs undergo glucose-regulated exocytosis and are capable of high turnover. Following kiss-and-run exocytosis, the SLMV RRP may be reloaded with γ-aminobutyric acid and undergo several cycles of exo- and endocytosis. Our observations support a role for β-cell SLMVs in a synaptic-like function of rapid intra-islet signaling. Diabetes 54:736–743, 2005

Pancrėatic β-cells store insulin in large dense-core vesicles (LDCVs) but also contain nonpeptide neurotransmitters such as γ-aminobutyric acid (GABA), which is stored in small synaptic-like microvesicles (SLMVs) (1,2). Glucose-stimulated GABA release is a potentially important mechanism mediating glucose-dependent inhibition of glucagon secretion (3,4). However, most studies on GABA secretion from β-cells are difficult to interpret (5), and glucose has been suggested to either inhibit, stimulate, or have no effect on GABA release over 2–24 h (6–8). Recently, we reported depolarization and Ca\(^{2+}\)-evoked quantal GABA release using a receptor-based assay (9) and suggested that this resulted from exocytosis of SLMVs. In agreement with a previous report (10), we found by electron microscopy that LDCVs contained less GABA than the cytosol (9). However, it was recently reported that GABA is indeed present in LDCVs (11), and GABA can conceivably be co-released with insulin rather than through a separate exocytotic pathway. As such, it remains uncertain whether the SLMVs in β-cells are capable of regulated exocytosis.

Cell-attached capacitance measurements monitor the exocytosis of single vesicles as small as synaptic vesicles (12). Unlike traditional whole-cell capacitance techniques cell-attached measurements are performed on metabolically and electrically intact cells, allowing examination of glucose-stimulated exocytosis. Capacitance measurements can also be made on cell-free excised-patch capacitance measurements to examine whether β-cell SLMVs undergo regulated exocytosis and whether this occurs in parallel with the exocytosis of LDCVs. Such knowledge is required to establish whether the SLMVs participate in rapid glucose-dependent paracrine signaling within islets.

We now report that SLMVs do undergo regulated exocytosis in both INS-1 insulinoma and primary rat β-cells. We further demonstrate that, like LDCVs, exocytosis of SLMVs is glucose-dependent and coupled to the activity of L-type Ca\(^{2+}\) channels. The RRP of SLMVs is proportionally larger and released more quickly than the RRP of LDCVs and SLMVs often recycled rapidly at the plasma membrane by kiss-and-run exocytosis. Our observations therefore support a role for β-cell SLMVs in rapid glucose-dependent intra-islet signaling.

RESEARCH DESIGN AND METHODS

Cell culture. INS-1 cells, a gift from Dr. C. Wollheim (Geneva, Switzerland), were cultured as described previously (14) and plated in 35-mm plastic dishes 18–48 h before capacitance measurements. Islets from female Sprague Dawley rats (200 g) were isolated and dispersed to single cells essentially as described for mouse islets (15). Cells were plated on poly-L-lysine-coated 35-mm plastic dishes 3–24 h before capacitance measurements.

Electron microscopy. INS-1 cells were harvested and fixed in 2.5% glutaraldehyde for 1 h at 4°C, treated with 1% osmium tetroxide, dehydrated, and embedded in Durcupan (Sigma-Aldrich, Stockholm, Sweden). Ultra-thin sections (70–90 nm) were put on Cu-grids and contrasted with uranyl acetate and lead citrate before examination by electron microscopy (JEM 1200; JEOL-USA, Peabody, MA). Analysis of vesicle diameters and calculation of vesicle density were performed as described elsewhere (16). Total vesicle number was determined by multiplying the vesicle density by the average INS-1 cell volume determined by confocal microscopy. Three separate preparations were performed.

From 1Section of Diabetes, Metabolism and Endocrinology, Lund University, Lund, Sweden; and 2Oxford Centre for Diabetes, Endocrinology and Metabolism, Churchill Hospital, University of Oxford, Oxford, U.K.

Address correspondence and reprint requests to Dr. Patrick E. MacDonald, Lund University, Molecular and Cellular Physiology, Tornavägen 10, BMC B11, 221 84 Lund, Sweden. E-mail: patrick.macdonald@mphy.lu.se.

Received for publication 18 August 2004 and accepted in revised form 13 December 2004.

GABA, γ-aminobutyric acid; LDCV, large dense-core vesicle; RRP, readily releasable pool; SLMV, synaptic-like microvesicle.

© 2005 by the American Diabetes Association.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
were analyzed, and because there was no difference between these, data were pooled.

**Capacitance measurements.** Capacitance was measured using the cell-attached and excised patch configurations (12,13). The sine wave output (25 kHz, 50 mV rms) of an SR830 dual-phase lock-in amplifier (Stanford Research Systems, Sunnyvale, CA) was connected to the external stimulus input of an EPC9 amplifier (HEKA Elektronik, Lambrecht/Pfalz, Germany) and applied to membrane patches around 0 mV. The current monitor output of the EPC9 (filter 1–30 kHz, 20 mV/pA gain) was connected to the signal input of the SR830 with a 1:10 voltage divider (20 dB attenuator). Auto C-fast compensation was used, and occasional readjustment prevented clipping of the output signal. The X and Y outputs of the SR830 lock-in were low-pass filtered at 3 ms (24 dB) and connected to A/D inputs of the EPC9 for data acquisition at 10 kHz by Pulse software (HEKA Elektronik). Online calibration of the lock-in phase angle was performed using pure capacitance increases produced by pulses of pipette suction.

For cell-attached recordings of INS-1 and rat β-cells, fire-polished patch pipettes had average resistances of 2.49 ± 0.4 and 2.32 ± 0.5 MΩ, respectively. The bath solution contained (in mmol/l) 125 NaCl, 4 KCl, 2 CaCl2, 0 or 10 glucose, and 10 HEPES at pH 7.2. The adenylate cyclase activator forskolin (5 μmol/l) was also included when indicated. The pipette solution contained (in mmol/l): 125 NaCl; 4 KCl; 10, 2.6, or 0 CaCl2; 0 or 2 EGTA; 1 MgCl2; 13 TEA-Cl; and 10 HEPES at pH 7.2. In some experiments, the Ca2+ channel blockers isradipine (2 μmol/l, Alomone Labs, Jerusalem, Israel) or SNX-482 (100 nmol/l; Peptide Institute, Osaka, Japan) were also included in the pipette solution. With isradipine, the final DMISO concentration was 0.05%

For excised-patch recordings, the average pipette resistance was 3.6 ± 0.04 MΩ (n = 11). The patch pipette was withdrawn from the cell following seal formation while applying slight suction. Brief (~1 s) exposure to air by removal of the patch from the bath was used to ensure that inside-out patches, rather than microspheres, were formed at the pipette tip (17). The bath solution for excised patch recordings contained (in mmol/l) 120 Na-glutamate, 20 NaCl, 5 KCl, 1 MgCl2, 0.1 EGTA, and 10 HEPES at pH 7.4. The pipette solution and stimulation buffer were the same as the bath solution except these contained 0.5 and 2 mmol/l CaCl2, respectively, and no EGTA. For all experiments, the bath was perfused continuously at 32°C.

**Off-line analysis.** Small-phase offsets were corrected offline when necessary with custom software created in MatLab (v. 6.5.0; The MathWorks, Natick, MA). The phase angle was adjusted as follows, where X and Y are the transformed outputs and θ is the phase adjustment:

\[ X_t = X \cdot \cos \theta + Y \cdot \sin \theta \] (1)

\[ Y_t = -X \cdot \sin \theta + Y \cdot \cos \theta \] (2)

An average phase adjustment of 5.96 ± 0.26° was applied to 23.3% of the cell-attached events. All subsequent data analyses were performed with Origin 6.0 (Microcal Software, Northampton, MA). Data were baseline subtracted from an INS-1 cell stimulated with 10 mmol/l glucose, where stepwise changes in capacitance (Im/2πf) represent exocytosis of single vesicles. Small (at arrow) and large capacitance steps from β shown in expanded scale following a 4.65° phase correction. These likely represent exocytosis of an SLMV and LDCV, respectively.

**RESULTS**

**Exocytosis of single vesicles in membrane patches.** Whole-cell capacitance measurements (Fig. 1A, left) have been used extensively to investigate exocytosis in β-cells (20) but lack the sensitivity to resolve fusion of single vesicles. Cell-attached (Fig. 1A, right) capacitance measurements were performed on metabolically intact INS-1 and rat β-cells. The two outputs of the lock-in amplifier reflect Re and Im, which correspond to patch conductance and capacitance (Im/2πf), respectively (Fig. 1B). Unitary capacitance steps reflecting exocytosis of single vesicles were observed in cell-attached patches of INS-1 and rat β-cells. Although online phase calibration generally allowed for good separation of the conductance compo-
nents, small offsets can be observed as changes in \( \text{Re} \) during a step in \( \Delta \text{Im}/2 \pi f \). These were corrected offline, and single corrected steps are shown on expanded scale in Figs. 1 \( C \) (INS-1) and \( D \) (primary rat \( \beta \)-cell). Downward steps in capacitance were also observed, reflecting exocytosis (not shown).

**SLMVs and LDCVs observed by electron microscopy.** To explain the ultrastructural origin of the capacitance steps, INS-1 cells were analyzed by electron microscopy (Fig. 2A). Profile diameter histograms of clear and dense-core vesicles are presented in Figs. 2 \( B \) and \( C \). The clear vesicles had a mean diameter of \( 81 \pm 2 \) nm (\( n = 96 \)). The profile distribution of the dense-core vesicles (\( n = 926 \)) was best fit with two Gaussian distributions with average diameters of \( 115 \pm 4 \) and \( 171 \pm 13 \) nm, suggesting two groups of LDCVs. Assuming a spherical geometry, fusion of a single SMLV is predicted to increase capacitance by \( 371 \) and \( 827 \) aF, respectively (Fig. 2 \( D \)). The intracellular density of LDCVs and SLMVs, respectively, with average diameters of \( 347 \) and \( 91 \) nm (9).

**SLMV and LDCV exocytosis is glucose and calcium-dependent.** Glucose dependence is an essential feature of the exocytotic release of insulin (20), while it remains to be determined whether exocytosis of SLMVs is similarly regulated. The histogram of exocytotic events from INS-1 cells in the absence of glucose is shown in Fig. 3A (79 events, 41 cells, 197.7 min). Exocytosis of both large and small vesicles was stimulated with 10 mmol/l glucose (Fig. 3B; 152 events, 48 cells, 192.7 min). The distribution of events was fit with two Gaussian distributions (black lines). The small-diameter (\( 129 \pm 2 \) aF; an estimated diameter of \( 67 \pm 1 \) nm) group corresponded with SLMV values obtained by electron microscopy. The large-diameter group averaged \( 841 \pm 68 \) aF (\( 185 \pm 17 \) nm diameter), corresponding to the large-LDCV group observed by electron microscopy. The small-diameter group of LDCVs observed by electron microscopy did not undergo exocytosis and may therefore represent a population of immature granules. In the absence of glucose, SLMVs were slightly larger (\( 177 \pm 5 \) aF, 79 \( \pm 2 \) nm, \( P < 0.001 \)), while the average size of LDCVs was unchanged.

In the absence of glucose, the rates of SLMV and LDCV exocytosis were \( 0.16 \pm 0.03 \) and \( 0.22 \pm 0.06 \) vesicles \( \cdot \) patch\(^{-1} \cdot \) min\(^{-1} \). These values equate to whole-cell rates of 25.4 and 34.9 vesicles \( \cdot \) cell\(^{-1} \cdot \) min\(^{-1} \) (Fig. 3D) (research design and methods). Glucose stimulation caused a 1.6- and 2.4-fold increase in the rates of SLMV and LDCV exocytosis, respectively (Fig. 3D). With 10 mmol/l glucose, removal of Ca\(^{2+} \) (Fig. 3C; 79 events, 43 cells, 207.8 min) decreased SLMV and LDCV exocytosis to the basal level observed in the absence of glucose (Fig. 3D).

**SLMV and LDCV exocytosis couples to \( \alpha \)-type calcium channels.** It is well established that exocytosis of insulin-containing LDCVs is preferentially coupled to Ca\(^{2+} \) entry through \( \alpha \)-type channels (21,22). \( \beta \)-type channels mediate >60% of the non-\( \alpha \)-type Ca\(^{2+} \) current in mouse \( \beta \)-cells (22) and may regulate insulin secretion from INS-1 (23,24) and mouse \( \beta \)-cells (22,25). We therefore investigated the role of these channel types in exocytosis of single SLMVs and...
LDCVs in INS-1 cells. Because experiments were performed with Ca\(^{2+}\) present only in the pipette (and absent from the bath), Ca\(^{2+}\) can only enter the cell via channels within the membrane patch.

With glucose present, inclusion of the L-type Ca\(^{2+}\) channel antagonist isradipine (2 \(\mu\)mol/l) in the pipette solution reduced LDCV exocytosis by 49\% \((P < 0.01; 87\) events, 50 cells, 230.9 min) (Fig. 4A). A similar effect was observed for SLMV exocytosis (58\% reduction; \(P < 0.001\)). Exocytotic rates in the presence of isradipine were not different from those in the absence of glucose (Fig. 4C). The R-type Ca\(^{2+}\) channel antagonist SNX-482 (100 \(\mu\)mol/l; 111 events, 42 cells, 165.3 min) had no significant effect on LDCV or SLMV exocytosis (Figs. 4B and C). Therefore, both SLMVs and LDCVs are primarily coupled to Ca\(^{2+}\) entry through L-type rather than R-type channels.

**Kiss-and-run exocytosis of SLMVs.** The proportion of SLMV content undergoing exocytosis in response to glucose (6\% per minute) is larger than for LDCVs (1% per minute). Thus, SLMV turnover is expected to occur rapidly. We therefore examined the occurrence of capacitance flickers resulting from transient fusion of vesicles in cell-attached membrane patches of INS-1 cells. Under the present conditions, capacitance flickers consistent with LDCV kiss-and-run were not observed in INS-1 cells. In contrast, 21\% of SLMV exocytosis occurred as kiss-and-run in the presence of glucose (Fig. 5A). This was not dramatically different in the absence of glucose (29\%) or extracellular Ca\(^{2+}\) (29\%), or with elevated (10 \(\mu\)mol/l) pipette Ca\(^{2+}\) (27\%). The identity of these vesicles as SLMVs was confirmed by the distribution of flicker sizes (average of 144 ± 9 aF, 71 ± 4 nm diameter) (Fig. 5B). Linear regression demonstrates a strong correlation between the exocytotic up-step and the endocytotic down-step, and the slope of this relationship approached unity (Fig. 5C), suggesting the vesicles were recaptured intact.

In the presence of glucose, the duration of SLMV kiss-and-run events increased from 146 ± 28 ms \((n = 14)\) with 2.6 mmol/l Ca\(^{2+}\) in the patch pipette to 581 ± 89 ms \((n = 20, P < 0.001)\) with 10 mmol/l Ca\(^{2+}\). Complete removal of Ca\(^{2+}\) did not decrease the mean lifetime of SLMV kiss-and-run (207 ± 63 ms, \(n = 10)\) beyond that observed at 2.6 mmol/l Ca\(^{2+}\). Paradoxically, removal of glucose also lengthened the duration of kiss-and-run events to 440 ± 138 ms \((n = 12, P < 0.05)\). We further examined the kinetics of SLMV kiss-and-run by plotting cumulative histograms of flicker duration (Figs. 5D and E). Curves were best-fit with a double exponential function, the two components of which may represent different conformational states of the kiss-and-run fusion pore (26). With 10 mmol/l Ca\(^{2+}\) in the pipette, both time-constants were slowed without drastically altering their relative contribution (Fig. 5D), while removal of glucose (Fig. 5E) shifted events away from the faster state (from 64 to 38\%) and toward the slower state (from 36 to 62\%). Therefore, the effects of 10 mmol/l Ca\(^{2+}\) and removal of glucose on SLMV kiss-and-run duration differ mechanistically, suggesting a distinct glucose-derived signal promoting a more rapid reinternalization of these vesicles.

**Stimulation of SLMV exocytosis in rat β-cells by cAMP.** Exocytosis of SLMVs was examined in cell-attached patches of rat β-cells stimulated with 10 mmol/l glucose (115 events, 46 cells, 196.6 min) (Fig. 6A). Twenty-nine percent of these occurred as kiss-and-run (Fig. 6B). The rate of glucose-stimulated SLMV exocytosis (0.30 ± 0.03 vesicles · patch\(^{-1}·\)min\(^{-1}\)) was increased 1.6-fold \((P < 0.05)\) by 5 \(\mu\)mol/l forskolin (194 events, 66 cells, 297.2 min), although the relative proportion of flickering vesicles was unchanged (Fig. 6C). The distribution of flickering vesicle sizes (115 ± 6 aF; 62 ± 1 nm diameter) confirmed that SLMVs underwent kiss-and-run, and linear regression suggests these were recaptured intact (Fig. 6C). Forskolin increased the mean flicker duration from 376 ± 34 (\(n = 14)\) to 746 ± 69 ms (\(n = 23, P < 0.05)\), which is associated with slowing of the fast and slow kinetic components (Fig. 6D).

**SLMV and LDCVs in cell-free membrane patches.** To examine the contribution of the physically docked RRP to Ca\(^{2+}\)-dependent exocytosis of SLMVs and LDCVs, experiments were performed on cell-free excised membrane patches (Fig. 7A) of INS-1 cells, where vesicles mobilized from the cell interior make little or no contribution to exocytosis. Exocytosis was elicited by application of Ca\(^{2+}\) to the intracellular surface of the patch via a puffer pipette. Exocytosis was rarely observed without stimulation (5 events, 11 patches, 18.5 min) (Fig. 7B, traces 1–4) and increased fourfold upon Ca\(^{2+}\) application (20 events, 11 patches, 17.7 min) (Fig. 7B, traces 5–8). The rates of SLMV
and LDCV exocytosis during repeated Ca\(^{2+}\) pulses are shown in Fig. 7C. Cumulative histograms were fit to a single exponential function to determine RRP size and the time-constant of depletion (Fig. 7D). The SLMV and LDCV RRRPs consisted of 99 and 67 vesicles, respectively. The SLMV RRP was depleted more quickly (\(\tau = 4.6\) s) than the LDCV RRP (\(\tau = 9.3\) s), and initial rates of exocytosis were 17.8 and 6.5 vesicles/s. In these experiments, a higher proportion (67\%) of SLMV exocytosis occurred as transient (duration 604 ± 68 ms, \(n = 8\)) vesicle fusions, suggesting that the SLMV RRP recycles preferentially by kiss-and-run (Fig. 7E).

**DISCUSSION**

LDCVs of endocrine cells contain peptides or amines that target distant tissues and/or mediate effects over relatively long periods. The role of endocrine SLMVs is not entirely clear. \(\beta\)-Cell SLMVs are proposed to mediate glucose-dependent GABAergic paracrine inhibition of glucagon secretion (3,4). However, regulated exocytosis of SLMVs has not been unequivocally demonstrated. The significant noise reduction achieved with the cell-attached configuration improves the resolution of membrane capacitance measurements by ~100-fold (27) and allows detection of single SLMVs and LDCVs. Here we consider, in turn, 1) whether SLMVs and LDCVs are similarly regulated by glucose-dependent Ca\(^{2+}\) entry through t-type channels, 2) whether SLMVs undergo kiss-and-run exocytosis, and 3) whether the SLMVs can be subdivided into functional pools with variable release competence. Finally, we discuss the physiological implications of our findings.

**Glucose and Ca\(^{2+}\) dependence of LDCV and SLMV exocytosis.** We find that exocytosis of GABA-containing SLMVs is regulated by glucose-dependent Ca\(^{2+}\) entry, in agreement with recent studies demonstrating GABA secretion from single \(\beta\)-cells in response to Ca\(^{2+}\) infusion or depolarization (3,9). We also report that, like insulin-containing LDCVs (21), SLMV exocytosis is closely linked to Ca\(^{2+}\) entry through t-type channels, as indicated by the inhibitory action of isradipine. We estimate the glucose-stimulated rate of LDCV exocytosis at 1% per minute of the total granule content, reasonably close to previous estimates (0.2–0.3% per minute) (28). Importantly, both values are much lower than that derived for exocytosis of SLMVs (6% per minute). Exocytosis of SLMVs therefore proceeds in parallel with LDCVs, and this is consistent with a role for GABA in glucose-dependent paracrine inhibition of glucagon secretion.

The high rates (25 and 35 vesicles \(\cdot\) cell\(^{-1}\) \(\cdot\) min\(^{-1}\)) of
SLMV and LDCV exocytosis observed in the absence of glucose or Ca\(^{2+}\) is consistent with basal release of GABA from rat β-cells (29) and of insulin, which is often relatively high in insulinoma cells (as much as 4–5% per 30 min of total content [30]). Although the basal exocytosis we observe may represent constitutive exocytotic processes (31), there is an alternative explanation pertaining to the experimental conditions used (no extracellular Ca\(^{2+}\)). When extracellular Ca\(^{2+}\) is low, the voltage-dependent Ca\(^{2+}\) channels become permeable to Na\(^+\) (32), activating an inward current at normal resting potentials. The resulting cellular depolarization may spread into the patch membrane, opening Ca\(^{2+}\) channels under the Ca\(^{2+}\)-containing pipette and leading to localized Ca\(^{2+}\) influx. This is consistent with the strong reduction of exocytosis when Ca\(^{2+}\) was excluded from the pipette solution and also indicates the important role for local, rather than global, Ca\(^{2+}\) levels (33).

Kiss-and-run exocytosis of SLMVs. LDCV kiss-and-run has been observed using cell-attached capacitance measurements in bovine and rat chromaffin cells (13,34,35), human neutrophils (36), and rat posterior pituitary nerve terminals (37). The proportion of LDCV kiss-and-run in those studies varied between 2 and 13%, although 78% was reported with 90 mmol/l external Ca\(^{2+}\). While insulin-containing LDCVs may remain structurally intact following exocytosis (38,39), recent reports in agreement with the present data demonstrate that the majority of β-cell LDCV exocytosis occurs via complete vesicle fusion (40–42). By contrast, a large percentage (as much as 30% in intact cells) of exocytosed SLMVs were recycled rapidly by recapture of the vesicle. Our observation that these are recaptured largely intact is consistent with studies of lipophilic dye loss from synaptic vesicles (43). The same vesicles can thus be expected to undergo multiple cycles of exocytosis and endocytosis. When coupled with vesicular uptake of GABA (1), this provides a means of rapidly refilling the pool of neurotransmitter-containing vesicles. Such a mechanism is clearly not possible for refilling of LDCVs with insulin, and the lipid membrane can only be recycled via the trans Golgi network.

It was recently proposed that vesicles undergoing kiss-and-run exist in several states corresponding to different time-constants of fusion pore closure (26), and Ca\(^{2+}\) (within a certain range) and forskolin shifted transiently fusing vesicles into slower kinetic states. In agreement, we do indeed observe at least two kinetic states (τ\(_1\) and τ\(_2\)) for kiss-and-run corresponding to the fast and slow time constants (Figs. 6 and 7), and LDCV flicker times were increased two- to fourfold by high Ca\(^{2+}\) and forskolin. Functionally, this may increase the amount of GABA released during transient fusion pore opening under these strong stimulatory conditions. Thus, GABA secretion may be increased to a greater extent under these conditions than is indicated simply by the number of SLMVs undergoing exocytosis.

Rapid release of the SLMV RRP. Secretory vesicles are classified according to their release competence (20). Most vesicles belong to an intracellular reserve pool and are unable to directly undergo exocytosis. A fraction of vesicles, namely the RRP, are capable of immediate exocytosis when intracellular Ca\(^{2+}\) rises to stimulatory levels. By our estimates, 15% of SLMVs and 1% of LDCVs are docked and primed for release in INS-1 cells. Not only is the SLMV RRP proportionally larger than the LDCV RRP, it is depleted more rapidly, as indicated by the faster time-constant and initial rate of RRP release, consistent with a role for β-cell SLMVs in fast paracrine signaling (44). Exocytosis of the docked SLMV RRP was associated with a relatively high occurrence (67%) of kiss-and-run. This appears to be a property of the SLMV RRP rather than the result of the strong Ca\(^{2+}\) stimulus used in the excised patch experiments, since there is no evidence in cell-attached measurements that stronger stimuli (high Ca\(^{2+}\) or forskolin) increase kiss-and-run exocytosis. In addition, we find that the LDCV RRP in INS-1 cells is greater than our previous estimate (15 vesicles) (14), which was based on an inaccurate estimate of the unitary capacitance of a single LDCV. Having now measured the unitary capacitance of INS-1 LDCVs, we can revise our previous estimate to 74 vesicles. This is strikingly close to our separate

FIG. 7. Exocytosis of the SLMV and LDCV RRPs in excised membrane patches from INS-1 β-cells. A: Inside-out excised-patch capacitance measurements. B: A series of consecutive 10-s capacitance (Im/2πf) traces from an excised patch. No stimulus was applied in traces 1–4. The break in trace 1 resulted from pipette suction applied for calibration purposes. In traces 5–8, Ca\(^{2+}\) buffer was applied to the intracellular surface of the membrane (at arrows). C: Rate of SLMV and LDCV exocytosis versus stimulus number. D: Cumulative histograms of SLMV and LDCV exocytosis, fit to exponential functions [N(1 – e\(^{-\tau}\))], where N is the number of vesicles in the RRP and τ is the RRP depletion time-constant. For the SLMVs, N and τ were estimated as 99 vesicles and 4.6 s, respectively. The corresponding values for the LDCVs were 67 vesicles and 9.3 s. E: Examples of Ca\(^{2+}\)-stimulated SLMV kiss-and-run from different membrane patches, seen as transient capacitance increases (marked a–d). In some patches Ca\(^{2+}\) also triggered separate endocytotic (downward) steps. **P < 0.01 and ***P < 0.001 compared with the first stimulus unless otherwise indicated.
calculation (67 vesicles) based on RRP depletion in the excised patch experiments. 

**Physiological implications.** This study provides the first direct measurements of Ca\(^{2+}\), and glucose-induced SLMV exocytosis in insulin-secreting cells, which is the most likely route for release of GABA from the \(\beta\)-cells. This reinforces previous (indirect) arguments that GABA-containing SLMVs do undergo regulated exocytosis (9). We recently reported that GABA modulates insulin secretion from the \(\beta\)-cell in an autocrine fashion by activation of GAB\(\text{A}\)\(\beta\) receptors (45), accounting for a significant portion of the glucose-dependent inhibition of glucagon secretion through the activation of \(\alpha\)-cell \(G\text{ABA}\) receptors (3). The ability of cAMP to stimulate SLMV exocytosis may help to reconcile the conundrum that whereas cAMP stimulating hormones like glucagon-like peptide-1 suppress glucagon secretion in intact islets, they actually stimulate exocytosis in isolated \(\alpha\)-cells (46). Given the present data, we propose that glucagon-like peptide-1 suppresses glucagon secretion indirectly by enhancing the release of GABA-containing SLMVs from \(\beta\)-cells and that this effect overrides the direct stimulatory action on \(\alpha\)-cell exocytosis.

Both the present and previous (9) studies indicate that regulation of LDCVs and SLMVs share many features. It, however, remains possible that there are undocumented differences, such that GABA can be released without concomitant insulin secretion. This could explain our recent observation that glucagon secretion is inhibited at glucose concentrations associated with little insulin release (47). It is also worth considering that LDCVs contain a number of substances in addition to insulin (20). It is possible that the objective of having different routes of exocytosis in \(\beta\)-cells is not only to separate insulin and GABA secretion, but also to allow differential secretion of low-molecular-weight compounds.

**ACKNOWLEDGMENTS**

Financial support was from the Swedish Strategic Foundation; the Göran Gustafsson Foundation for Research in the Natural Sciences and Medicine; the Swedish Research Council (grants 8647 and 13147); the Novo Nordisk Foundation; the Albert Påhlssons Foundation; and the Faculty Council (grants 8647 and 13147); the Novo Nordisk Foundation; the Swedish Research Council; and the Faculty Council (grants 8647 and 13147).

The authors thank Dr. Manfred Lindau (Cornell) for advice regarding patch capacitance measurement.

**REFERENCES**