SUMOylation Regulates Insulin Exocytosis Downstream of Secretory Granule Docking in Rodents and Humans

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OBJECTIVE—The reversible attachment of small ubiquitin-like modifier (SUMO) proteins controls target localization and function. We examined an acute role for the SUMOylation pathway in downstream events mediating insulin secretion.

RESEARCH DESIGN AND METHODS—We studied islets and β-cells from mice and human donors, as well as INS-1 832/13 cells. Insulin secretion, intracellular Ca2+, and β-cell exocytosis were monitored after manipulation of the SUMOylation machinery. Granule localization was imaged by total internal reflection fluorescence and electron microscopy; immunoprecipitation and Western blotting were used to examine the soluble NSF attachment receptor complex formation and SUMO1 interaction with synaptotagmin VII.

RESULTS—SUMO1 impairs glucose-stimulated insulin secretion by blunting the β-cell exocytotic response to Ca2+. This is rapid and does not require altered gene expression or insulin content, is downstream of granule docking at the plasma membrane, and is dependent on SUMO-conjugation because the deSUMOylating enzyme, sentrin/SUMO-specific peptidase (SENP)-1, rescues exocytosis. SUMO1 coimmunoprecipitates with the Ca2+ sensor synaptotagmin VII, and this is transiently lost upon glucose stimulation. SENP1 overexpression also disrupts the association of SUMO1 with synaptotagmin VII and mimics the effect of glucose to enhance exocytosis. Conversely, SENP1 knockdown impairs exocytosis at stimulatory glucose levels and blunts glucose-dependent insulin secretion from mouse and human islets.

CONCLUSIONS—SUMOylation acutely regulates insulin secretion by the direct and reversible inhibition of β-cell exocytosis in response to intracellular Ca2+ elevation. The SUMO protease, SENP1, is required for glucose-dependent insulin secretion.

SUMOylation also regulates membrane proteins, including K+ channels (11–14), nonselective cation channels (15), and kainate receptors (16), and is suggested to control Ca2+ influx at nerve terminals (17). Membrane proteins are critical to insulin secretion from β-cells. After translocation from the cell interior, secretory granules are docked to the plasma membrane by formation of a soluble NSF attachment receptor (SNARE) protein complex in conjunction with Munc18a (18–20). This complex interacts closely with the voltage-dependent Ca2+ channels (21,22). Ca2+ sensing at the site of exocytosis is mediated, at least in part, by synaptotagmin VII (23,24). An interaction between the SUMOylation and exocytotic pathways, and the impact of this interaction on glucose-dependent insulin secretion, has not been fully explored.

In the present work, we find that SUMO1 impairs glucose-stimulated insulin secretion but not insulin content or Ca2+ responses. Rather, SUMOylation directly inhibits the β-cell exocytotic response to membrane depolarization or infusion of Ca2+, an effect that is acute, not requiring altered gene expression, and reversible by the SUMO protease SENP1. This inhibitory effect is downstream of granule docking at the plasma membrane. A role for SUMO1 in downstream Ca2+-dependent exocytosis is further suggested by its interaction with synaptotagmin VII. This association is transiently lost upon glucose-stimulation but returns within 30–60 min, correlating with glucose-dependent exocytosis. Overexpression of SENP1 prevents interaction of SUMO1 with synaptotagmin VII and augments exocytosis at low glucose and after prolonged glucose stimulation. Finally, knockdown of the deSUMOylating enzyme inhibits β-cell exocytosis and glucose-dependent insulin secretion from mouse and human islets. Thus, SUMO1 impairs insulin exocytosis, possibly through a direct interaction with the exocytotic machinery, and the deSUMOylating enzyme SENP1 is required for glucose-dependent insulin secretion.

RESEARCH DESIGN AND METHODS

Cells and cell culture. Human embryonic kidney (HEK)-293 cells were cultured in DMEM with 10% FBS, 100 units/mL penicillin, and 100 μg/mL streptomycin at 37°C and 5% CO2. INS-1 832/13 cells (a gift from Dr. Christopher Newgard, Duke University) were cultured in RPMI-1640 containing 11.1 mmol/L glucose and supplemented with 10% FBS, 10 mmol/L HEPES, 2 mmol/L l-glutamine, 1 mmol/L sodium pyruvate, 50 μmol/L β-mercaptoethanol, and 100 units/mL penicillin/streptomycin at 37°C and 5% CO2. Islets from male C57/LBL6 mice were isolated by collagenase digestion. Mouse islets and cells were cultured in RPMI with l-glutamine, 10% FBS, and 100 units/mL streptomycin.

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Received 30 March 2010 and accepted 8 December 2010.

Diabetes Publish Ahead of Print, published online January 24, 2011

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penicillin/streptomycin. Human islets and cells were cultured in low-glucose (1 g/L) DMEM with l-glutamine, 110 mg/L sodium pyruvate, 10% FBS, and 100 units/mL penicillin/streptomycin. All studies were approved by the animal care and use committee and the human research ethics board at the University of Alberta.

**Constructions, adenosurmines, and recombinant peptides.** The human SUMO1-YFP (25) in the pEYFP-C1 vector (Clontech, Palo Alto, CA) was a gift from Dr. Heidi McBride (University of Ottawa). The human SUMO1, Ubc9, and SENP1 constructs were in the pcMV6-XL4 vector and were from Origene Technologies (Rockville, MD). The pIRE6-EGFP vector (Clontech) or pcDNA3.1 were used as transcriptional reporter vectors in recombinant human islets from GenEx Tex (San Antonio, TX); SENP1 and Ubq enzymes were from Enzo Life Sciences (Plymouth Meeting, PA). Glutathione S-transferase (GST) was from Sigma-Aldrich Canada (Oakville, Canada). Recombinant adenosurmines expressing green fluorescent protein (GFP) (Ad-GFP), SUMO1 (Ad-SUMO1), or SENP1 (Ad-SENP1) were created using pAdtrackCMV and the AdEasy system (www.coloncancer.org). Three separate SENP1-targeted small-hairpin RNA (shRNA) constructs and a scrambled control were designed against identical human and mouse sequences using Genscript siRNA target-finder software (Genscript, Piscataway, NJ). These were synthesized as hairpins oligos with BomiIII and HinvIII restriction sites on the 5′ and 3′ ends and ligated into the pRNAT-1H.1/shuttle vector. Knockdown was tested by quantitative real-time PCR and Western blot, followed by the production of the recombinant adenosurmin (Ad-shSENP1 and Ad-shScramble) in HEK-290 cells using the Adeno-X Expression System 1 (Clontech).

**Insulin secretion measurements.** Insulin secretion measurements were performed at 37°C in Krebs ringer buffer (KRB) (in mmol/L: 115 NaCl, 5 KCl, 1.2 MgCl₂, 25 NaHCO₃, and 10 HEPES, pH 7.4). Twenty-five islets per group were preincubated for 2 h in 1 mL of glucose KRB then for 1 h in KRB at 1 mmol/L glucose followed by 1 h with 16.7 mmol/L glucose. INS-1 832/13 cells were cultured overnight in RPMI with 5 mmol/L glucose then dialyzed with a control GST peptide (2 μmol/mL) displayed reduced islet insulin content (data not shown) or impaired intracellular Ca²⁺ responses to glucose (n = 6) (Fig. 1B), suggesting that the inhibitory effect lies downstream of Ca²⁺ entry.

To examine whether acute SUMOylation affects the downstream mechanism of insulin secretion, recombinant proteins were dialyzed directly into mouse β-cells for ~4 min via a patch-clamp pipette (Fig. 1C). Exocytosis was then measured as the whole-cell capacitance response to a train of membrane depolarizations (Fig. 1D-F), β-Cells dialyzed with a control GST peptide (2 μmol/mL) displayed robust exocytosis (n = 41), whereas recombinant SUMO1 (2 μmol/mL) blunted the response by 85% (P < 0.001, n = 19). This required SUMO conjugation because it could be readily reversed by codialysis of the SUMO protease SENP1 (6 μg/mL; n = 19).

**SUMOylation increases granule docking and SNAPRE complex formation.** A lack of effect of SUMO1 on the intracellular Ca²⁺ response, together with the acute inhibition of exocytosis by SUMO1, suggests a role in regulating downstream events in insulin secretion. Similar to mouse islets, glucose-stimulated insulin secretion also was impaired in INS-1 832/13 cells overexpressing SUMO1 (Fig. 2A). Dense-core granules were clearly seen to be morphologically docked at the plasma membrane by electron microscopy of cells overexpressing either GFP or SUMO1 (Fig. 2B; representative of 33 and 31 images, respectively). Indeed, membrane-associated secretory granule density, measured by TIRF microscopy, was increased by 70% in SUMO1-expressing (n = 50, P < 0.01) (Fig. 2C). This was normalized by a 4-h culture at 1 mmol/L glucose and increased again in SUMO1-expressing (n = 67) versus control (n = 57, P < 0.05) cells following a 15-min glucose stimulation (Fig. 2C).
cells transfected with SUMO1 (Fig. 2D). This could be partially reversed by coexpression of SENP1, whereas total SNARE protein levels were unchanged. Cells expressing GFP alone showed the glucose-stimulated disassembly of the complex consistent with the release of docked granules, but this was blunted after overexpression of SUMO1 (Fig. 2E). Some Munc18a may be pulled down with syntaxin-1A independent of the SNARE-complex. However, changes in Munc18a occurred in parallel with SNAP-25 and VAMP2, consistent with Munc18a participation in a “SNAREpin” structure (30). Therefore, SUMO1 does not impair insulin secretion by reducing insulin granule recruitment and docking at the plasma membrane. Inhibition of the secretory process by SUMO1 occurs downstream of the docking event.

**SUMOylation inhibits Ca^{2+}-dependent exocytosis.** We examined whether Ca^{2+}-dependent exocytosis, per se, is impaired by SUMO1. We thus examined voltage-dependent calcium channel (VDCC) function and capacitance responses in INS-1 832/13 cells overexpressing a conjugation-competent SUMO1-YFP (25) alone or together with SENP1. Upon depolarization of cells to 0 mV, the peak VDCC

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**FIG. 1. SUMOylation impairs glucose-stimulated insulin secretion by acute inhibition of β-cell exocytosis.** A: Insulin secretion from isolated mouse islets expressing either GFP alone (Ad-GFP; □) or SUMO1 (Ad-SUMO1; ■). B: Intracellular Ca^{2+} responses to glucose and KCl in these islets by ratiometric imaging of Fura-2-AM. The glucose-stimulated increase in the Fura-2 ratio is shown at the bottom. C: Single-cell experiments were performed on β-cells by whole-cell patch clamp, allowing the direct and acute intracellular dialysis of a control peptide (GST), SUMO1, or SENP1. D: Mouse β-cell exocytosis, measured as an increase in capacitance (cell size) during a train of ten 500-ms depolarizations (top), after ~4 min of dialysis with GST, SUMO1, or SUMO1 + SENP1. E: Average capacitance response to each step-wise depolarization. F: The total capacitance response over the train of depolarizations. *P < 0.05; ***P < 0.001 vs. controls.
current in control cells was $-20.9 \pm 2.9 \text{ pA/pF (} n = 30) and after SUMO1-YFP expression was $-31.3 \pm 4.6 \text{ pA/pF (} n = 16). This approached statistical significance (} P = 0.054), although it should be noted that an increase in Ca$^{2+}$ current cannot account for an inhibitor effect of SUMO1-YFP on the exocytotic response.

Insulin granules are physically coupled to VDCCs (31), and loss of this interaction can reduce insulin secretion and β-cell exocytosis (32). To examine VDCC-granule coupling, cells were subjected to depolarizations of increasing duration (Fig. 3A and B). Short pulses allow local Ca$^{2+}$ signals, whereas longer pulses mediate increases in bulk cytosolic Ca$^{2+}$ (21,33). Compared with GFP alone (} n = 14), expression of SUMO1-YFP impaired the exocytotic response to even the longest (~2.5 s) depolarization (inhibited by 76%; } n = 16, } P < 0.01). This was rescued by coexpression of SENP1 (} n = 14). This suggests that decoupling of secretory granules from VDCCs does not

FIG. 2. SUMOylation does not impair secretory granule docking. A: Insulin secretion from INS-1 832/13 cells expressing either GFP alone (Ad-GFP, □) or SUMO1 (Ad-SUMO1, ■). B: Representative electron micrographs of INS-1 832/13 cells expressing either GFP alone (Ad-GFP) or SUMO1 (Ad-SUMO1). Scale bar represents 1 μm. C: Membrane-localized secretory granules (labeled with IAPP-mCherry) imaged by TIRF microscopy of INS-1 832/13 cells expressing SUMO1 or control vector. Representative images obtained under standard culture conditions (left). Average secretory granule density at the plasma membrane (right) following standard overnight culture at 11.1 mmol/L glucose or 4-h culture at 1 mmol/L glucose followed by acute (15 min) stimulation at 10 mmol/L glucose. D: Munc-18a/SNARE complex formation in INS-1 832/13 cells expressing GFP, SUMO1, and/or SENP1, assessed by immunoprecipitation (IP) of syntaxin 1A (Syn-1A) and blotting for Munc-18a, Syn-1A, SNAP-25, and VAMP2 under standard culture conditions. E: Same as D but after a 2-h incubation at 1 mmol/L glucose and following 15 min at 15 mmol/L glucose (basal and stimulated). **$P < 0.01; *P < 0.01$ vs. GFP.
account for the impaired exocytotic response. Indeed, exocytosis could not be rescued by the direct infusion of 200 nmol/L free Ca\textsuperscript{2+} and remained impaired in cells expressing SUMO1-YFP (by 95%, \( n = 15, P < 0.001 \)) (Fig. 3C and D). Again, this was rescued by coexpression of SENP1 (\( n = 15 \)). The exocytotic response of INS-1 832/13 cells also was impaired by overexpression of the SUMOylating enzyme Ubc9 (by 90%, \( n = 7, P < 0.001 \)) rather than SUMO1 itself (Fig. 3E and F). Importantly, this was rapidly rescued by intracellular dialysis of SENP1 (6 \( \mu \)g/mL, \( n = 10 \)). Taken together, these results demonstrate that SUMOylation inhibits Ca\textsuperscript{2+}-dependent exocytosis distal to granule docking and Ca\textsuperscript{2+} entry.

**SUMOylation inhibits exocytosis in human \( \beta \)-cells.** We next examined the effect of upregulating SUMOylation in human \( \beta \)-cells positively identified by insulin immunostaining. Similar to the effect observed in the INS-1 832/13 cells, overexpression of SUMO1-YFP inhibited the exocytotic response of human \( \beta \)-cells elicited both by infusion of 200 nmol/L free Ca\textsuperscript{2+} (by 94%, \( n = 13 \) and 5 from two

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**FIG. 3.** SUMOylation impairs Ca\textsuperscript{2+}-dependent exocytosis. A: Exocytotic capacitance responses to depolarizations of increasing duration (10, 320, and 2560 ms shown) of INS-1 832/13 cells expressing GFP, SUMO1-YFP, or SUMO1-YFP + SENP1. B: Average capacitance responses from A plotted vs. depolarization duration. C: The whole-cell capacitance response of INS-1 832/13 cells measured during direct application of 200 nmol/L free Ca\textsuperscript{2+} via the patch pipette. D: The average capacitance response of cells expressing GFP, SUMO1-YFP, or SUMO1-YFP + SENP1 at 200 s following initiation of Ca\textsuperscript{2+} infusion and normalized to initial cell size. E: Endogenous and overexpressed Ubc9 in INS-1 832/13 cells (left). Exocytotic capacitance responses (right) in INS-1 832/13 cells expressing GFP or Ubc9 and dialyzed with either GST or SENP1. F: Average cumulative capacitance responses over the series of 10 depolarizations. **P < 0.01; ***P < 0.001 vs. GFP.
SUMOylation inhibits exocytosis in human β-cells. A: The exocytotic response of single human β-cells to direct intracellular dialysis of 200 nmol/L free Ca<sup>2+</sup> measured as increased cell capacitance. B: The average capacitance response, at 200 s following Ca<sup>2+</sup> infusion and normalized to initial cell size, in human β-cells expressing GFP, SUMO1, or SUMO1 + SENP1. C: Exocytosis elicited by a series of ten 500-ms depolarizations in human β-cells expressing GFP, SUMO1, or SUMO1 + Ubc or SUMO1 + SENP1. D: Average capacitance responses to each depolarization (left) and the total capacitance response over the train (right). E: Exocytotic responses of human β-cells expressing GFP or the SUMO-conjugating enzyme Ubc9 alone. F: Average capacitance responses to each depolarization (left) and the total capacitance response over the train (right). ***P < 0.001 vs. GFP alone.
distal to Munc18a/SNARE complex assembly, we postulated that SUMO1 might interact with synaptotagmin VII, a likely mediator of exocytotic Ca\textsuperscript{2+}-sensing in \(\beta\)-cells (23,24) that possesses a potential type II nonconsensus SUMOylation site (34). We find that SUMO1 coimmunoprecipitates with synaptotagmin VII, and vice versa, from INS-1 832/13 cells and human islets (Fig. 5C–H). SUMO1 was not pulled down with synaptotagmin IX (data not shown), which also is reported to contribute to the control of Ca\textsuperscript{2+}-dependent exocytosis in \(\beta\)-cells (35,36). The association with synaptotagmin VII was SUMOylation-dependent because it could be enhanced by overexpression of Ubc9 or SUMO1 (Fig. 5D and H) and could be prevented by SENP1 (Fig. 5D). Furthermore, the endogenous SUMO1/synaptotagmin VII interaction is transiently disrupted after glucose stimulation (Fig. 5B–G) in both the INS-1 832/13 cells (\(n = 3\)) and human islets (\(n = \) five donors). This generally occurred at 15 min poststimulation (although in human islets this was occasionally observed at 30 min as shown in Fig. 5G). Additionally, in one of five human donors the glucose-dependent loss of this interaction was not observed; islets from this donor displayed neither exocytosis nor glucose-stimulated insulin secretion (data not shown).

**SUMOylation and glucose-dependent \(\beta\)-cell exocytosis.** Because glucose stimulation transiently disrupts the SUMO1/synaptotagmin VII interaction, we considered whether the SUMOylation pathway is an important determinant of the glucose dependence of \(\beta\)-cell exocytosis. We examined whether SUMOylation acts to limit Ca\textsuperscript{2+}-dependent exocytosis at low glucose and after prolonged glucose stimulation, conditions where we observed a strong SUMOylation-dependent interaction between SUMO1 and synaptotagmin VII. Initially, preincubation of cells at 1 mmol/L glucose for 4 h was followed by acute (15 min) exposure to either 1 or 10 mmol/L glucose (Fig. 6A).
The exocytic response of mouse β-cells at 1 mmol/L glucose was low (Fig. 6B and C, ●) and was significantly enhanced in response to 10 mmol/L glucose (Fig. 6B and C, ○). At 1 mmol/L glucose, the direct and intracellular dialysis (6 μg/mL; mouse, n = 40, P < 0.001) or overexpression (human, n = 38 from six donors, P < 0.001) of the deSUMOylating enzyme SENP1 was sufficient to enhance exocytosis but had no further effect at 10 mmol/L glucose (□). The total capacitance response under these conditions (right). C: Same as in B but human β-cells by overexpression rather than acute dialysis. Also, expression of the SUMO ligase, Ubc9, prevented glucose enhancement of exocytosis in these cells (△). **P < 0.01; ***P < 0.001 vs. 1 mmol/L glucose control.

**FIG. 6. SUMOylation and deSUMOylation enzymes modulate glucose enhancement of exocytosis in mouse and human β-cells. A: Cells were pre-incubated at 1 mmol/L glucose for 4 h prior to a 15-min exposure to either 1 or 10 mmol/L glucose, after which exocytosis was measured as the whole-cell capacitance response to a series of membrane depolarizations (arrow). B: Exocytosis in mouse β-cells was blunted at 1 mmol/L (●), and enhanced at 10 mmol/L (○) glucose in cells dialyzed with GST. Dialysis of SENP1 peptide at 1 mmol/L glucose recapitulated the effect of glucose stimulation (■), but had no further effect at 10 mmol/L glucose (□). The total capacitance response under these conditions (right). C: Same as in B but human β-cells by overexpression rather than acute dialysis. Also, expression of the SUMO ligase, Ubc9, prevented glucose enhancement of exocytosis in these cells (△). **P < 0.01; ***P < 0.001 vs. 1 mmol/L glucose control.**

SENP1 is required for glucose-stimulated exocytosis and insulin secretion. To investigate whether SENP1 is required in glucose- and depolarization-stimulated exocytosis and insulin secretion, we used an shRNA strategy to knock down this deSUMOylating enzyme. Our recombinant adenovirus expressing SENP1 shRNA (Ad-shSENP1)
knocked down the endogenous enzyme (58.3 ± 17.2%, n = 3) measured by Western blot (Fig. 8A). The exocytotic response of mouse β-cells was impaired by 65 and 95%, at 48 and 66 h, respectively, after infection with Ad-shSENP1 (n = 14–18, P < 0.001) (Fig. 8A). Similar results were observed in human β-cells, where knockdown of SENP1 impaired exocytosis by 87% at 66 h (n = 17–26 from three donors, P < 0.001) (Fig. 8B). The inhibitory effect of SENP1 knockdown could be rapidly rescued by intracellular dialysis with recombinant SENP1 (6 μg/mL) in both human (n = 12) (Fig. 8C) and mouse (n = 8) (data not shown) β-cells. Furthermore, SENP1 knockdown completely prevented the ability of glucose to enhance mouse β-cell exocytosis (n = 16, P < 0.001) (Fig. 8D) and blunted glucose-stimulated insulin release from both mouse (n = 3) (Fig. 8E) and human (n = three donors, P < 0.05) (Fig. 8F) islets.

**DISCUSSION**

SUMOylation regulates transcription factor targeting and function, including that of MafA and pancreatic and duodenal homeobox-1 in pancreatic β-cells (7,8). More recently, extranuclear roles for SUMO have emerged, including the regulation of mitochondrial and ion channel function (11–15,25,37), which implicate SUMOylation in the acute control of cellular function without requiring altered gene transcription. Here, we demonstrate that SUMOylation
Acutely and reversibly controls glucose-dependent insulin secretion in rodents and humans through the regulation of Ca\textsuperscript{2+}-dependent exocytosis downstream of secretory granule docking at the plasma membrane. Furthermore, the deSUMOylating enzyme SENP1 augments β-cell exocytosis and is required for glucose-dependent insulin secretion.

Posttranslational SUMOylation is suggested to regulate key plasma membrane proteins, including ion channels (11–15) and receptors (16), although in one case this has been questioned (38). We showed previously that overexpression of SUMO1 in INS-1 and human β-cells inhibits voltage-dependent K\textsuperscript{+} (\(K\text{v}\)) currents and modulates excitability (13). However, in contrast with the expected insulinotropic effect associated with \(K\text{v}\) inhibition (28,39), SUMO1 overexpression impaired glucose-stimulated insulin secretion. It is possible that mitochondrial function is impacted by SUMO1 (25,40). However, that SUMO1 primarily inhibits insulin secretion through a downstream mechanism is suggested by 1) the lack of effect on intracellular Ca\textsuperscript{2+} responses, 2) the inability of direct Ca\textsuperscript{2+} infusion to stimulate exocytosis after SUMO1 overexpression, and 3) the rapidity at which direct intracellular SUMO1 dialysis blocks exocytosis. Furthermore, although SUMOylation modulates transcription factor function in many cell types, including β-cells (7,8), altered gene expression or reduced insulin content is not required for the inhibitory effect of SUMO1 on β-cell exocytosis. This is evidenced again by the ability of acute SUMO1 infusion to inhibit exocytosis, as well as the lack of effect of SUMO1 on SNARE protein expression and preserved insulin content.

SUMO1 exerts the majority of its effects via its covalent and reversible conjugation to target proteins (41). SUMO conjugation is indeed required for SUMO1 to inhibit β-cell exocytosis because the SUMO protease, SENP1, rescues the exocytotic response. A role for endogenous SUMOylation in insulin exocytosis is demonstrated by manipulation of the SUMO conjugation and protease machinery. Upregulation of the SUMO ligase, Ubc9, or knockdown of the SUMO protease, SENP1, inhibits β-cell exocytosis, both of which are rescued by dialysis with SENP1. Thus, SUMOylation acutely and reversibly inhibits exocytosis in human and rodent β-cells.

The point at which SUMOylation inhibits β-cell exocytosis lies downstream of insulin granule trafficking to the plasma membrane and physical docking, as indicated by our electron microscopy and TIRF studies and the abundant Munc18a/SNARE complex assembly following SUMO1 overexpression. The ubiquitin/proteosome pathway is implicated in the regulation of β-cell voltage-dependent Ca\textsuperscript{2+} channels (42), and SUMOylation is reported to modulate Ca\textsuperscript{2+} influx at presynaptic nerve terminals (17). However, SUMOylation impairs β-cell exocytosis at the point of Ca\textsuperscript{2+} triggering of membrane fusion rather than Ca\textsuperscript{2+} entry or Ca\textsuperscript{2+} channel/granule coupling, which is most clearly shown by an inability of direct Ca\textsuperscript{2+} application to elicit exocytosis after SUMO1 upregulation. Consistent with
an effect on exocytotic Ca\(^{2+}\) sensing, we find that endogenous SUMO1 interacts with synaptotagmin VII.

Synaptotagmin VII represents a major exocytotic Ca\(^{2+}\) sensor in β-cells (23,24). The SUMO1-positive band at 75 kDa pulled down with synaptotagmin VII is consistent with synaptotagmin VII splice variants (SYTVIIα or β) expressed in β-cells (43) and corresponds to the synaptotagmin VII–positive band pulled down with a SUMO1 antibody. Additional work is required to determine whether synaptotagmin VII is SUMOylated directly, perhaps at its predicted nonconsensus SUMOylation site, or whether the coimmunoprecipitated protein is a SUMOylated binding partner. Certainly, the SUMO1/synaptotagmin VII interaction, whether direct or indirect, is SUMOylation dependent because it is upregulated by Ubc9 and lost upon SENP1 overexpression.

The SUMO1/synaptotagmin VII interaction is transiently lost upon glucose stimulation and returns within 30–60 min. Similarly, SUMOylation of MafA in β-cells increases at low glucose (7). Although the mechanism(s) underlying these effects remains to be elucidated, the present data suggest a key role for (de)SUMOylation in glucose-dependent insulin secretion. This is supported by our findings that SENP1 disrupts the SUMO1/synaptotagmin VII interaction and mimics the ability of glucose to enhance exocytosis. Furthermore, SENP1 knockdown demonstrates that this deSUMOylating enzyme is required for β-cell exocytosis and glucose-stimulated insulin secretion, and upregulation of Ubc9, which prevents dissociation of SUMO1 from synaptotagmin VII, prevents glucose-dependent exocytosis. Finally, we find that restoration of the SUMO1/synaptotagmin VII interaction correlates with the suppression of exocytosis during longer glucose stimulations, and robust exocytosis is restored by SENP1, demonstrating that SUMOylation as a limiting factor under this condition. Although it is tempting to speculate that the time course of SUMOylation effects on exocytosis relates to secretory capacity during first- and/or second-phase insulin secretion, it is clear that additional work is required to delineate the exact temporal relationship between SUMOylation and insulin secretion.

We conclude that the SUMOylation pathway plays an important and acute role in glucose-dependent insulin secretion in rodents and humans via the acute and dynamic regulation of Ca\(^{2+}\)-dependent exocytosis. At low glucose, and following prolonged glucose stimulation, SUMOylation acts as a “brake” to prevent the Ca\(^{2+}\)-induced exocytotic release of insulin. Conversely, the deSUMOylating enzyme, SENP1, augments Ca\(^{2+}\)-dependent exocytosis and is required for robust glucose-stimulated insulin secretion.

**ACKNOWLEDGMENTS**

Funding for this research was provided by operating grants from the Canadian Institutes of Health Research to P.E.M. (MOP-97845) and H.Y.G. (MOP-86544). X.-Q.D. is supported by a fellowship from the Alberta Heritage Foundation for Medical Research (AHFMR). C.H. is supported by the Gladys Wirtanen nie Woodrow Studentship from the Alberta Diabetes Foundation. P.E.M. holds scholarships from the Canadian Diabetes Foundation and AHFMR and is the Canada Research Chair in Islet Biology. No potential conflicts of interest relevant to this article were reported.

X.-Q.D. and G.P. researched data and contributed to discussion. M.C., Y.K., and C.H. researched data. H.Y.G. contributed to discussion and reviewed and edited the manuscript. J.E.M.F. researched data and contributed to discussion. P.E.M. contributed to discussion and wrote the manuscript.

The authors are grateful to Nancy Smith (University of Alberta) for technical assistance, to Dr. Chris Newgard (Duke University) for providing INS-1 832/13 cells, and to Dr. James Shapiro and the Clinical Islet Laboratory at the University of Alberta for human donor islets.

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