SUMO downregulates GLP-1-stimulated cAMP generation and insulin secretion

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gon-like peptide-1 (GLP-1)-based incretin therapy is becoming central to the treatment of type 2 diabetes. Activation of incretin hormone receptors results in rapid elevation of cAMP followed by enhanced insulin secretion. However, the incretin effect may be significantly impaired in diabetes. The objective of this study is to investigate downregulation of GLP-1 signaling by small ubiquitin-related modifier protein (SUMO). Mouse islets exposed to high glucose showed increased expression of endogenous SUMO transcripts and its conjugating enzyme Ubc-9. Overexpression of SUMO-1 in mouse isletoma 6 (MIN6) cells and primary mouse β-cells resulted in reduced static and real-time estimates of intracellular cAMP upon receptor stimulation with exendin-4, a GLP-1 receptor (GLP-1R) agonist. GLP1-R was covalently modified by SUMO. Overexpression of SUMO-1 attenuated cell surface trafficking of GLP-1R, which resulted in significantly reduced insulin secretion when stimulated by exendin-4. Partial knock down of SUMO-conjugating enzyme Ubc-9 resulted in enhanced exendin-4-stimulated insulin secretion in mouse islets exposed to high glucose. Thus, SUMO modification of the GLP-1R could be a contributing factor to reduced incretin responsiveness. Elucidating mechanisms of GLP-1R regulation by sumoylation will help improve our understanding of incretin biology and of GLP-1-based treatment of type 2 diabetes.

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DNA Constructs, cDNA Preparation, and Quantitative RT-PCR

Human SUMO-1 with hemagglutinin (HA), mCherry, and GFP tags and human GLP-1R with HA and GFP tags were cloned into the expression vector pCDNA 3.1(+) (Invitrogen, La Jolla, CA). RNA from mouse isletoma (MIN6) cells and mouse islets were prepared with an RNasefree Mini Kit (Qiagen, Valencia, CA), and cDNA was prepared from 500 ng RNA by Superscript III Reverse transcriptase (Invitrogen). Primer pairs for quantitative RT-PCR were purchased from Origene (Rockville, MD). Quantitative RT-PCR was performed using Fast SYBR Green reagent using a Step one instrument (Applied Biosystems, Carlsbad, CA).

The fold change in expression of SUMO transcripts in islets exposed to 16 mM glucose was calculated by normalizing to the levels obtained from islets maintained in 5 mM glucose. The significance of the normalized fold change after a step increase in glucose was calculated by Student’s t-test using Graphpad prism software. The housekeeping gene, β-actin was used as an internal control.

Cell Culture, Islet Preparation, and Transfection

MIN6 cells were grown in DMEM supplemented with 15% FBS, 100 IU/µl penicillin, and 100 ng/ml streptomycin, and MIN6 cells stably expressing GFP-SUMO were grown in DMEM supplemented with 15% FBS and 500 ng/ml G418 (Invitrogen, Carlsbad, CA). Mouse islets were prepared following an approved protocol by the University of Chicago Institutional Animal Care and Use Committee. Islets were isolated from pancreata of 8- to 10-wk-old C57BL/6J wild-type mice (Jackson Laboratory, Bar Harbor, ME) using collagenase P (Roche Diagnostics, Basel, Switzerland) digestion and a Ficoll gradient. Islets were trypsinized, and the primary cells were cultured.
in RPMI 1640 medium supplemented with 10% FBS, 2 mM l-glutamine, 100 IU/ml penicillin, and 100 mg/ml streptomycin. MIN6 cells and isolated primary cells were transfected with Lipofectamine 2000 according to the manufacturer’s instructions (Invitrogen).

**Coimmunoprecipitation**

Immunoprecipitation experiments were designed to detect noncovalent and covalent interactions between GLP-1R and SUMO-1. For the detection of noncovalent interaction with SUMO and GLP-1R, MIN6 cells stably expressing GFP-SUMO-1 and transiently expressing HA-tagged GLP-1 receptor (GLP-1R-HA) were lysed in RIPA buffer (Santa Cruz Biotechnology, Santa Cruz, CA) and immunoprecipitated with a rabbit monoclonal antibody raised against HA epitope, YPYDVPDYA from influenza hemagglutinin (Cell Signaling Technology, Boston, MA).

GLP-1R-HA and GFP-SUMO-1 were detected by mouse monoclonal antibody raised from influenza hemagglutinin (Cell Signaling Technology, Boston, MA) and rabbit polyclonal antibody raised against GFP (Invitrogen).

**Cell-Surface Biotinylation**

For cell-surface biotinylation, MIN6 cells cotransfected with IgD epitope (Fig. 2). Briefly, for the detection of dynamic changes in cAMP on agonist stimulation, MIN6 cells and mouse islets were cotransfected with SUMO-1 fused to the red fluorescent protein mCherry and driven by rat insulin promoter 2 along with Epac-camps. Control cells were transfected with Epac-camps and mCherry vector alone. Live cells were imaged 48 h posttransfection in KR2 buffer on a Nikon inverted epifluorescence microscope with a charge-coupled device camera. About 12–15 cells were found to express Epac-camps, and 6–10 cells/35-mm glass bottom dish were found to express both fluorescent proteins, indicating the β-cells. Pancreatic β-cells from multiple 35-mm dishes were selected, and fluorescence was recorded before and during stimulation with 100 nM exendin-4 for 3 h. The supernatant was collected, and the cells were lysed in RIPA buffer. Insulin content in the supernatant and lysate was calculated from MIN6 cells stably expressing GFP-SUMO-1 and control cells expressing empty vector by a sensitive mouse insulin ELISA kit (ALPCO, Salem, NH). The cells were plated in 35-mm dishes and stimulated with 100 nM exendin-4 for 3 h. The supernatant was collected, and the cells were lysed in RIPA buffer. Insulin content in the supernatant and lysate was calculated according to the manufacturer’s instructions (Invitrogen).

**Insulin ELISA and Partial Knock Down of Ubc-9**

Insulin content was calculated from MIN6 cells stably expressing GFP-SUMO-1 and control cells expressing empty vector by a sensitive mouse insulin ELISA kit (ALPCO, Salem, NH). The cells were plated in 35-mm dishes and stimulated with 100 nM exendin-4 for 3 h. The supernatant was collected, and the cells were lysed in RIPA buffer. Insulin content in the supernatant and lysate was calculated according to the manufacturer’s protocol.

**Data Analysis**

The statistical analysis was carried out by Graphpad Prism software.

**RESULTS**

**Mouse Islets Exposed to High Glucose Show Increased Expression of SUMO and Ubc-9 Transcripts**

Expression of the components of the SUMO pathway is upregulated by various environmental stress conditions such as osmotic, hypoxic, heat, oxidative, and genotoxic stresses (36). Here, we investigated whether chronic exposure of islets to high glucose elicits a similar response. Mouse islets incubated in 16 mM glucose for 48 h showed increased expression of SUMO-1 (6.4-fold), SUMO-2 (4.3-fold), SUMO-3 (1.7-fold), and Ubc-9 knock down was accomplished using a validated shRNA vector (Origene). Retroviral particles were generated in HEK-292 cells according to the manufacturer’s instructions (Clontech, Mountain View, CA). Four constructs were tested for efficiency in MIN6 cells, and one showing ~50% knock-down efficiency was selected. Dispersed mouse islets were infected for 16 h and cultured in 16 or 5 mM glucose for 24 h. The islet cells were stimulated with 16 mM glucose and 50 nM exendin-4 for 2 h, and secreted and cytosolic insulin was quantified by ELISA (ALPCO).

**Enhanced Expression of SUMO-1 Downregulates GLP-1R**

Activation of the GLP-1R signaling pathway stimulates adenylyl cyclase, resulting in an increase in cytosolic cAMP.
showed that both, in insulinoma cells and primary/H9252 were transfected with GFP or GFP-tagged SUMO-1. The cells with control cells using a cAMP-specific ELISA. MIN-6 cells extendedin-4 did not cause an increase in cAMP. overexpressing SUMO-1, stimulation of GLP-1 receptor by
mCherry-tagged SUMO-1 (Fig. 2, and YFP fused to the cAMP-binding domain of the exchange protein Epac-2 (Epac-camps) was used to measure spatial and temporal changes in cAMP signaling (17). The Epac biosensor undergoes conformational changes upon cAMP binding that result in loss of FRET, measured as an increase in FRET ratio. MIN6 cells expressing endogenous GLP-1R were transiently cotransfected with Epac-camps and mCherry-tagged SUMO-1 or mCherry vector. Cells expressing both proteins were selected, and the FRET ratio was recorded after addition of 100 nM extendedin-4. Similarly, dissociated primary mouse β-cells were transfected with Epac-camps and mCherry-SUMO-1 or mCherry alone as control, driven by rat insulin promoter-2 to ensure β-cell-specific expression. Addition of 100 nM extendedin-4 caused a fivefold increase in the FRET ratio in MIN6 cells expressing mCherry compared with cells expressing mCherry-tagged SUMO-1 (Fig. 2, A and C). Mouse primary β-cells expressing mCherry showed a 3.7-fold increase in FRET ratio in response to extendedin-4 compared with cells expressing mCherry-SUMO-1 (Fig. 2, B and C). This result shows that both, in insulinoma cells and primary β-cells overexpressing SUMO-1, stimulation of GLP-1 receptor by extendedin-4 did not cause an increase in cAMP.

Next, we assessed total cAMP produced by extendedin-4 treatment in MIN6 cells overexpressing SUMO-1 and compared it with control cells using a cAMP-specific ELISA. MIN-6 cells were transfected with GFP or GFP-tagged SUMO-1. The cells were sorted by FACS 48 h postransfection and treated with 100 nM extendedin-4. Control cells expressing GFP alone showed a 1.9-fold increase in total cAMP concentration, whereas cells expressing GFP-tagged SUMO showed only a 1.3-fold increase following extendedin-4 treatment (Fig. 2D). A similar response was seen when extendedin-4 treatment was done in the presence of phoshodiesterase inhibitor IBMX despite slight elevation in basal cAMP compared with cells not treated with IBMX. This assay confirmed that enhanced expression of SUMO-1 resulted in significantly reduced cAMP response following agonist treatment and that SUMO-mediated downregulation of GLP-1R signaling is independent of phoshodiesterase activity (Fig. 2D). We next investigated whether SUMO-1 directly modified the GLP-1 receptor.

SUMO-1 Binds Covalently and Noncovalently to the GLP-1 Receptor

Although SUMO is known to interact noncovalently with some proteins, noncovalent interaction was shown to be required for efficient E3 SUMO ligase activity (15, 23). To detect noncovalent interaction, COOH-terminally tagged GLP-1R-HA was expressed in MIN6 cells and coimmunoprecipitated with an anti-HA antibody. A double band corresponding to GFP-SUMO-1 coimmunoprecipitated with the GLP-1R-HA protein, demonstrating the presence of a noncovalent interaction between the two proteins (Fig. 3A). To investigate whether SUMO-1 covalently interacts with GLP-1R, we transfected MIN6 cells with 1d4 epitope-tagged GLP-1R and GFP-SUMO-1 or with a conjugation-deficient GFP-SUMO1AG construct where the last four amino acids containing the diglycine motif were deleted. GLP-1R-1d4 was coimmunoprecipitated with 1d4 antibody under denaturing conditions as described in MATERIALS AND METHODS. A higher-molecular-weight band that corresponds to GFP-SUMO-modified GLP-1R was detected with anti-GFP antibody in the presence of GFP-SUMO-1, but not GFP- or conjugation-deficient SUMO, indicating a likely covalent modification of GLP-1R by SUMO-1 (Fig. 3B).

The direct covalent modification of GLP-1R by SUMO in live cells was confirmed by FRET analysis by expressing GLP-1R-CFP and YFP-SUMO-1. Nonspecific interactions between CFP and YFP-SUMO were not observed, and this assay has extensively been used in several previous studies (3, 27, 34, 35). MIN6 cells transfected with GLP-1R-CFP and YFP-SUMO-1 or YFP-SUMO-1ΔGG were imaged 24 h postransfection. FRET was calculated by CFP (donor) bleaching as previously described (27, 28). The presence of a FRET interaction causes CFP to bleach more slowly, thus increasing the time constant (tau) compared with the non-FRET control. Analysis of the fluorescence decay curve shows a twofold increase in the time constant (tau) for CFP-GLP-1R only when YFP-SUMO-1 is present. Taken together, these two experiments support the conclusion that GLP-1R is directly modified by SUMO-1.

Enhanced Expression of SUMO-1 Impairs Cell Surface Trafficking of GLP-1R

Because SUMO-1 expression attenuated GLP-1R signaling upon agonist stimulation, we investigated the mecha-
nism that underlies SUMO-mediated loss of GLP-1R function. Three lines of evidence indicate that SUMO-1 interferes with the cell surface targeting of GLP-1R. First, we cotransfected GLP-1R fused with GFP at the COOH-terminus (GLP-1R-GFP) with mCherry-SUMO-1 in MIN6 cells. Confocal microscopy images showed apparent membrane localization of GLP-1R-GFP cotransfected with free mCherry vector (Fig. 4, A, C, E, and F). However, when GLP-1R-GFP was cotransfected with mCherry-SUMO, GFP fluorescence was found to be predominantly intracellular (Fig. 4, B, D, E, and F). To confirm these results, we used another GLP-1R construct with an HA epitope introduced after the signal peptide at the extracellular NH2-terminus (HA-GLP-1R) and cotransfected MIN6 cells with GFP or GFP-SUMO-1. The cells were fixed but not permeabilized and immunostained with anti-HA antibody to detect GLP-1R at the plasma membrane. Whereas HA antibody detected HA-GLP-1R at the membrane in cells transfected with GFP, very little or no HA-GLP-1R was observed in cells cotransfected with GFP-SUMO-1 (Fig. 4, G and H). These results were again confirmed by a cell surface biotinylation assay designed to detect the receptor at the cell surface. MIN6 cells were cotransfected with untagged SUMO or empty vector and the GLP-1R-GFP construct. Streptavidin-purified biotinylated membrane proteins were probed with an anti-GFP antibody to detect membrane-bound GLP-1R-GFP. The ratio of membrane vs. cytosol-associated GLP-1R was drastically reduced by fivefold in three independent experiments when the receptor was coexpressed with SUMO-1 (Fig. 5, A and B).

Fig. 2. Overexpression of SUMO-1 decreases cAMP response following agonist stimulation of glucagon-like peptide-1 receptor (GLP-1R). MIN6 cells and mouse primary islet cells were transfected with Epac-camps and mCherry-SUMO or mCherry vector. Dynamic changes in cAMP binding were measured as the change in the fluorescent resonance energy transfer (FRET) ratio. A: change in FRET ratio following exposure to 100 nM exendin-4 in MIN6 cells transfected with Epac-camps and mCherry (black) and Epac-camps and mCherry-SUMO (gray) when treated with 100 nM exendin-4. B: change in FRET ratio in mouse primary β-cells transfected with Epac-camps and mCherry (black) or mCherry-SUMO (gray) when treated with 100 nM exendin-4. C: graph showing mean change in FRET ratio in MIN6 cells. Overexpression of SUMO-1 caused 5- and 3.7-fold reduction in FRET ratio compared with the control in MIN6 cells and mouse primary β-cells, respectively. Error bars indicate means ± SD. Student’s t-test: ***P < 0.001; n = 6 cells from multiple dishes. D: total cAMP quantified by enzyme-linked immunosorbent assay (ELISA) after exendin-4 treatment of MIN6 cells transfected with green fluorescent protein (GFP) or GFP-SUMO. FAC-sorted GFP-SUMO cells showed only a 1.3-fold increase in total cAMP, whereas control GFP cells showed a 1.9-fold increase compared with untreated cells. A similar response of 1.8-fold increase was also seen in control cells when exendin-4 treatment was done in the presence of 100 µM IBMX, whereas SUMO-1-expressing cells showed only a 1.2-fold increase that is not statistically significant. Error bars indicate means ± SD; n = 6. Student’s t-test: P = 0.01–0.05 (*) and <0.0001 (****) for exendin-4-treated cells without IBMX and with IBMX, respectively.

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REGULATION OF GLP-1 RECEPTOR SIGNALING BY SUMO

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SUMO-1 binds both noncovalently and covalently to GLP-1R. 

**A**: MIN6 cells stably expressing GFP-SUMO-1 or GFP were transfected with hemagglutinin (HA)-tagged GLP-1 receptor (GLP-1R HA) and immunoprecipitated to detect protein interactions. To detect noncovalent interaction, cells were lysed without N-ethylmaleimide, immunoprecipitated with an anti-HA antibody, and detected with mouse GFP antibody. The arrow indicates an ~40-kDa double band representing GFP-SUMO-1. Lane 1, cell lysate GLP-1R HA + GFP; lane 2, cell lysate GLP-1R HA + GFP-SUMO-1; lane 3, immunoprecipitation (IP) from lane 1; lane 4, IP from lane 2; lanes 1–4, blotted with rabbit anti-HA antibody; lanes 5–8 are similar to lanes 1–4, blotted with mouse anti-GFP antibody.

**B**: to detect covalent interaction, MIN6 cells stably expressing GFP-SUMO-1 or GFP or conjugation-deficient GFP-SUMO-1 were transfected with GLP-1R 1d4, lysed, and immunopurified under denaturing conditions with anti-1d4 antibody, and the eluates were blotted with rabbit GFP antibody. Arrow indicates a ~90-kDa immunoprecipitated band that represents GLP-1R covalently linked to GFP-SUMO-1 present only in the lane expressing GFP-SUMO-1 and GLP-1R 1d4. Lane 1, cell lysate GLP-1R 1d4 + GFP; lane 2, cell lysate GLP-1R 1d4 + GFP-SUMO-1; lane 3, GLP-1R 1d4 + GFP-SUMO-1 ΔGG; lane 4, IP from lane 1; lane 5, IP from lane 2; lane 6, IP from lane 3; lanes 1–6 are blotted with mouse anti-1d4; lanes 7–12 are the same as lanes 1–6 and blotted with rabbit anti-GFP antibody. C: FRET analysis by donor bleaching between GLP-1R-cyan fluorescent protein (CFP) and yellow fluorescent protein (YFP)-SUMO-1 shows interaction between GLP-1R and SUMO. Graph shows normalized fluorescent decay curves in cells expressing GLP-1R-CFP and YFP-SUMO-1 (gray) or GLP-1R-CFP and YFP-SUMO-1 ΔGG (black). Inset: image of representative cells expressing GLP-1R-CFP and YFP-SUMO-1 or YFP-SUMO-1 ΔGG (black). ***: Student’s t-test: ***P < 0.0007.
interferes with the cell surface trafficking of the receptor, causing decreased receptor density at the membrane.

Next, we tested whether partial knock down of Ubc-9 is able to rescue SUMO-mediated intracellular retention of GLP-1R. MIN6 cells transfected with GLP-1R-GFP and mCherry-SUMO-1 were transduced with retroviral particles expressing shRNA against Ubc-9. Reduced expression of Ubc-9 resulted in diminished nuclear mCherry-SUMO, and GLP-1R-GFP was predominantly localized at the plasma membrane (Fig. 6).

Overexpression of SUMO-1 Results in Reduced Insulin Content and Agonist-Stimulated Insulin Secretion

Transcription factors that are involved in insulin gene expression such as MafA and cleaved COOH-termini of ICA512 are targets of sumoylation (32). SUMO-1 was not found to affect insulin content when overexpressed by transient transfection (6). However, MIN6 cells stably expressing GFP-SUMO-1 showed a 6.3-fold reduction in total insulin content compared with control cells that express empty vector. Similarly, GFP-SUMO-1 stable cells also showed a 2.3-fold reduction in secreted insulin when stimulated by exendin-4 compared with control cells (Fig. 7, A and B). These results indicate that prolonged expression of SUMO-1 reduces insulin content and GLP-1R agonist-stimulated insulin secretion.

We tested the presence of an endoplasmic reticulum (ER) stress-induced gene “Chop” in GFP-SUMO-overexpressing and control GFP-expressing cells by RT-PCR. cDNA was prepared from MIN6 cells overexpressing GFP-SUMO-1 and untransfected cells. The presence of a 350-bp fragment of Chop cDNA was tested by RT-PCR. GFP-SUMO or untransfected MIN6 cells showed basal expression of Chop, whereas cells expressing GFP-tagged misfolded insulin mutant C96Y

Fig. 4. Intracellular retention of GLP-1R when coexpressed with SUMO-1. A: GLP-1R-GFP expressed with mCherry vector shows a predominant plasma membrane fluorescence of the GLP-1R-GFP. B: GLP-1R-GFP expressed with mCherry-SUMO-1 shows decreased plasma membrane localization consistent with enhanced intracellular retention. C: enlarged image of a representative cell expressing GLP-1R-GFP and mCherry. Vertical line represents the line chosen for fluorescence line scan. D: enlarged image of a cell expressing GLP-1R-GFP and mCherry SUMO-1 with the line chosen for the line scan. E: top, representative fluorescence line scan of GLP-1R-GFP with mCherry in cell C that shows distinct peaks. Bottom, fluorescence line scan of GLP-1R-GFP with mCherry-SUMO-1 that shows uniform distribution. F: mean fluorescence ratio of membrane to cytosol shows 2.2-fold increase, indicating enhanced membrane fluorescence when SUMO-1 is not present. Error bars indicate means ± SD; n = 6. Student’s t-test: ***p < 0.0007; n = 12–15 cells from multiple dishes. Average length of the line scan at the membrane and cytosol for calculating the ratio is 0.4–0.5 μm. G: GLP-1R-HA coexpressed with GFP shows predominant plasma membrane fluorescence of the GLP-1R signal, detected with anti-HA antibody in nonpermeabilized cells. H: MIN6 cells transfected with the HA-GLP-1R construct coexpressed with mCherry-SUMO-1 and stained with anti-HA antibody shows diminished plasma membrane fluorescence of GLP-1R-HA in nonpermeabilized cells. Scale bar = 5 μm.
Akita) showed enhanced Chop gene expression (29). This indicates that ER stress is unlikely the reason for attenuated insulin gene expression in MIN6 cells expressing GFP-SUMO.

Partial Knock Down of Ubc-9 Results in Improved Insulin Secretion

Next, we tested whether downregulation of the SUMO pathway by partial knock down of the SUMO-conjugating enzyme Ubc-9 improves agonist-induced insulin secretion in mouse islets. Islet cells cultured in 5 or 16 mM glucose were transduced with retroviral particles expressing shRNA against Ubc-9, and exenclaxin-stimulated insulin secretion was quantified. No significant change in secretion between sh-RNA-Ubc-9 transduced and control cells was observed in cells maintained in low glucose, whereas cells in high glucose expressing Ubc-9 shRNA showed significant increase in insulin secretion compared with the scrambled control. Thus, partial inhibition of the SUMO pathway is shown to rescue glucose-induced reduction in exendin-4-stimulated insulin secretion.

DISCUSSION

The incretin pathway has important pancreatic and extrapancratic effects but is impaired in type 2 diabetes. GLP-1R gene expression is downregulated in hyperglycemia, contributing to reduced β-cell incretin responses in a diabetic rodent model (37). In addition, islets from type 2 diabetic patients also showed diminished incretin responsiveness (13, 16, 25, 31, 33). Even though protein kinase C has been implicated in the reduced gene expression of incretin receptors in hyperglycemia (37), other mechanisms are likely to contribute to the lack of incretin responsiveness. In this report, we show elevated mRNA expression of three isoforms of SUMO and the SUMO-conjugating enzyme Ubc-9 on exposure of pancreatic islets to high-glucose conditions. Enhanced expression of SUMO-1 leads to downregulation of GLP-1 signaling when measured as a function of cAMP generation. In addition, elevated expression of SUMO-1 also causes reduction in total insulin content and GLP-1R agonist-stimulated insulin secretion.

The cellular conditions that cause an increase in sumoylation in pancreatic β-cells have not been well studied. The SUMO conjugation/deconjugation equilibrium is modified under various cellular stress conditions in other cell types (21, 36). SUMO-1 expression is upregulated in hypoxia, resulting in...
enhanced sumoylation of target proteins such as cAMP-response element-binding protein and hypoxia-inducible factor-1β (1, 4). Similarly, we found that RNA transcripts for SUMO and the SUMO-conjugating enzyme Ubc-9 are upregulated in mouse islets maintained in high glucose, indicating that expression of the SUMO pathway is upregulated when islets are exposed to high-glucose concentrations. Enhanced expression of the SUMO pathway is therefore likely to impair glucose- and incretin hormone-stimulated insulin secretion. Other proteins may also be regulated in this way in the β-cell. For example, SUMO protein was shown to inhibit the voltage-dependent K⁺ channel Kv2.1 that resulted in widening of β-cell action potentials and a decreased firing frequency, but inhibition of Kv2.1 might augment insulin secretion in mouse β-cells (5, 14). Similarly, SUMO-mediated upregulation of signaling pathways in cellular stress, such as the NF-κB transcription pathway, might be beneficial under certain circumstances (7). On the other hand, a recent report shows that SUMO-1 impairs glucose-stimulated insulin secretion by binding to synaptogamin VII and preventing exocytosis (6). We found that overexpression of SUMO-1 resulted in intraacellular retention of the GLP-1R that was associated with reduced receptor density at the cell membrane. Even though the role of SUMO in nucleocytoplasmic trafficking is well documented (11), how SUMO aids in forward trafficking to the plasma membrane is yet to be investigated. One possibility is that SUMO prevents GLP-1R oligomerization. Receptor oligomerization is essential for forward trafficking of the secretin...
family of class B G protein-coupled receptors, of which the GLP-1R is a member (20). SUMO modification was also shown to inhibit oligomerization of other proteins such as apoptosis signal-regulating kinase 1 and E26 leukemia (TEL) protein (18, 38). Furthermore, enhanced SUMO modification increases solubility of proteins as in the case of vaccinia virus protein (26). Thus SUMO modification may affect GLP-1R oligomerization, perhaps by a change in the solubility or binding ability of sumoylated proteins. The mechanism by which SUMO affects trafficking of GLP-1R could also be due to posttranslational modification of GLP-1R and concomitant effects on components of the trafficking machinery. Moreover, downregulation of GLP-1R signaling is likely due to a compound effect of direct SUMO modification of the GLP-1R and the possible modifications of the components of this pathway. For example, SUMO-mediated regulation of cAMP-specific phosphodiesterase-4D5 has been reported previously (19). Similarly, SUMO modification of transcription factors involved in insulin gene expression could also contribute to attenuation of exendin-4-stimulated insulin secretion (24, 32).

Incretin responses and its use in therapy are becoming central to the treatment of type 2 diabetes. Here, we describe sumoylation as a mechanism that regulates incretin responsiveness that could contribute to the reduction of the incretin effect in diabetes. A thorough understanding of the role of SUMO in incretin receptor regulation will help improve the efficacy of incretin-based therapy for diabetes.

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DISCLOSURES

The authors have no conflict of interest.

AUTHOR CONTRIBUTIONS

Author contributions: S. Rajan and L. H. Philipson conception and design of research; S. Rajan, J. Torres, and M. S. Thompson performed experiments; S. Rajan, M. S. Thompson, and L. H. Philipson analyzed data; S. Rajan, J. Torres, and L. H. Philipson interpreted results of experiments; S. Rajan and L. H. Philipson drafted manuscript; S. Rajan and L. H. Philipson edited and revised manuscript; S. Rajan and L. H. Philipson approved final version of manuscript.

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