Reduction of *Syndecan* Transcript Levels in the Insulin-Producing Cells Affects Glucose Homeostasis in Adult *Drosophila melanogaster*

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Signaling by direct cell–matrix interactions has been shown to impact the transcription, secretion, and storage of insulin in mammalian β cells. However, more research is still needed in this area. Syndecans are transmembrane heparan sulfate proteoglycans that function independently and in synergy with integrin-mediated signaling to mediate cell adhesion to the extracellular matrix. In this study, we used the model organism *Drosophila melanogaster* to determine whether knockdown of the *Syndecan* (*Sdc*) gene expression specifically in the insulin-producing cells (IPCs) might affect insulin-like peptide (ILP) production and secretion. IPCs of adult flies produce three ILPs (ILP2, ILP3, and ILP5), which have significant homology to mammalian insulin. We report that flies with reduced *Sdc* expression in the IPCs did not show any difference in the expression of *ilp* genes compared to controls. However, they had significantly reduced levels of the circulating ILP2 protein, higher circulating carbohydrates, and were less glucose tolerant than control flies. Finally, we found that IPCs-specific *Sdc* knockdown led to reduced levels of head *Glucose transporter1* gene expression, extracellular signal-regulated kinase phosphorylation, and reactive oxygen species. Taken together, our findings suggest a cell autonomous role for *Sdc* in insulin release in *D. melanogaster*.

Keywords: syndecan, glucose-induced insulin secretion, ERK, AKT

Introduction

SYNDECANS ARE CELL-SURFACE TRANSMEMBRANE proteins that have been conserved throughout metazoan evolution and are present on adherent cells (Chakravarti and Adams, 2006). They belong to the family of heparan sulfate proteoglycans that also include glycosylphosphatidylinositol-anchored glypicans and secreted proteoglycans found in the extracellular matrix (ECM) (Morgan *et al.*, 2007; Xian *et al.*, 2010). Although invertebrates have only one *Syndecan* (*Sdc*) gene and protein, there are four genes (*SDC1*, *SDC2*, *SDC3*, and *SDC4*) and corresponding proteins in vertebrates (Chakravarti and Adams, 2006). Structurally, however, all syndecans are composed by a core protein with extracellular attachment sites for glycosaminoglycan chains that mediate interactions with a wide array of ligands, including ECM components and soluble growth factors. The extracellular domain extends into a transmembrane domain and a highly conserved short cytoplasmic tail (Morgan *et al.*, 2007; Xian *et al.*, 2010). The latter binds to cytoskeleton proteins and

allows syndecans to function independently and in synergy with integrin-mediated signaling to mediate cell adhesion to the ECM (Morgan *et al.*, 2007; Bellin *et al.*, 2009; Xian *et al.*, 2010; Couchman *et al.*, 2015). Engagement of ECM proteins to integrins and/or syndecans triggers mechanical and biochemical alterations, such as activation of the focal adhesion kinase, phosphoinositide-3-kinase/Akt (PI3K/Akt), and Ras/extracellular signal-regulated kinase (ERK) cascade, which convey a signal from the cell membrane through the cytoskeleton to the nucleus (Bellin *et al.*, 2009; Provenzano and Keely, 2011). This leads to chromatin remodeling and gene expression changes that, in turn, reorganize and remodel the ECM compartment (Nelson and Bissell, 2006). Thus, a state of “dynamic reciprocity” exists between cells and their ECM, which plays a fundamental role in regulating tissue structure and function (Nelson and Bissell, 2006). Several studies in humans and rodents strongly suggest that the protein composition and dynamics of the ECM in several metabolic tissues play a critical role in the link between obesity and metabolic complications

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(Williams *et al.*, 2015). However, we still know little about the impact of alterations in expression of mediators of cell–ECM interaction, such as syndecans, on the maintenance of energy balance and homeostasis.

The fly *Drosophila melanogaster* is a powerful model for studying human metabolic regulation (Owusu-Ansah and Perrimon, 2014). The power of *Drosophila* lies not only in its well-characterized biology, low cost, and excellent genetic and genomic resources, but also in the fact that nutrient-sensing signaling pathways and regulatory mechanisms involved in energy homeostasis are highly conserved (Leopold and Perrimon, 2007). For instance, as in mammals, circulating sugar levels are regulated by insulin-like peptides (ILPs) that are secreted into the circulating hemolymph (Leopold and Perrimon, 2007). The *Drosophila* genome contains eight *ilp* genes: *ilp1* through *-8*. In adult flies, *ilp2*, *ilp3*, and *ilp5*, which show significant homology to mouse and human insulin, are expressed in a set of neurosecretory cells of the *pars intercerebralis* in the brain known as the insulin-producing cells (IPCs) (Nassel *et al.*, 2013). Among the *ilp* genes, *ilp2* is the most expressed in the IPCs, the most abundant ILP in circulation, and the closest homolog of human insulin (Nassel *et al.*, 2013). The IPCs of adult flies display several commonalities with the human β cells with respect to insulin release, relying on membrane depolarization and influx of calcium in response to glucose for release of the IPCs-derived ILPs (Kreneisz *et al.*, 2010; Barry and Thummel, 2016). Partial genetic deletion of IPCs in the adult fly produces phenotypes similar to those observed in human diabetic patients (Haselton and Fridell, 2010). These include increased circulating carbohydrate (Broughton *et al.*, 2008; Haselton *et al.*, 2010) and impaired ability to clear carbohydrate from circulation following food intake (Haselton and Fridell, 2010). Homologs of members of the insulin-signaling pathway are also found in *Drosophila* and function similar to their mammalian cognates (Garofalo, 2002; Teلمان, 2009).

We previously showed that adult *Sdc* mutant flies displayed reduced whole-body energy metabolism compared to controls (De Luca *et al.*, 2010). Following up on this observation we reported that knocking down *Sdc* specifically in the fat body (the insect equivalent of mammalian adipose tissue and liver) perturbed energy balance and metabolism (Eveland *et al.*, 2016). Compared to controls, fat body-specific *Sdc* knockdown flies also exhibited a significant decrease in phosphorylation levels of PI3K/Akt and ERK (Eveland *et al.*, 2016), suggesting that changes in cell survival signaling pathways may mediate the effect of reduced fat body *Sdc* on metabolism (Kuznetsov *et al.*, 2004). One additional finding in our initial study was that hypomorphic *Sdc* mutant flies had lower expression of head *ilp* genes than controls (De Luca *et al.*, 2010). In mammals, the interaction between ECM components and their cell surface receptors has been shown to impact the transcription, secretion, and storage of insulin (Kaido *et al.*, 2006). We, therefore, reasoned that deficiency of *Sdc* in the IPCs might also affect *Drosophila* ILP2 production. To test our idea, we used the GAL4/UAS-RNA interference (RNAi) approach (Duffy, 2002) to knockdown the expression of the *Sdc* gene specifically in the IPCs. The results of the present study provide initial evidence of a role for *Sdc* in the glucose-induced release of circulating ILP2 in adult *D. melanogaster*.

Materials and Methods

Drosophila strains

The UAS-*Sdc*-RNAi line (Dietzl *et al.*, 2007) was obtained from the Vienna Stock Center (Stock ID: v13322) and its knockdown efficiency was previously reported (Eveland *et al.*, 2016). The *w¹¹¹⁸* wild-type line, from which the UAS-*Sdc*-RNAi line was originally generated (<http://flystocks.bio.indiana.edu/Browse/VDRCTb.htm>), was obtained from the Bloomington Stock Center and used as control line. The *ilp2-Gal4* line was a generous gift from Dr. Eric Rulifson (University of California, San Francisco). The *w¹¹¹⁸; ilp2-Gal4/+; UAS- Sdc-RNAi/+* (hereinafter referred to as *ilp2>Sdc-RNAi*) and *w¹¹¹⁸; ilp2-Gal4/+; +/+* (hereinafter referred to as *ilp2>w¹¹¹⁸*) genotypes originated from a cross between *ilp2-Gal4* males and UAS-*Sdc-IR* or *w¹¹¹⁸* female flies. All flies were reared in temperature-controlled incubators at 25°C and 60–75% relative humidity on a standard cornmeal, molasses, and yeast medium with 12 h light-dark cycles. Newly enclosed male flies were collected as virgins and aged 4–6 days before being assessed for the phenotypic assays described below.

Quantitative polymerase chain reaction

Flies were snap-frozen in liquid nitrogen between 9:00 and 10:00 am on collection days and stored at -80°C . Total RNA was extracted from fly heads using the Qiagen RNeasy Mini column filtration kit (Qiagen, Valencia, CA) following the manufacturer's protocols. cDNA was then made using the First Strand Synthesis kit (Invitrogen, Carlsbad, CA). Quantitative polymerase chain reactions (qPCRs) were performed using the protocol described in Jumbo-Lucioni *et al.* (2012).

Enzyme-linked immunosorbent assay

The ILP2 antibody was a generous gift from Dr. Ernst Hafen (Institute of Molecular Systems Biology, ETH Zurich). Hemolymph samples were collected using the protocol described in Park *et al.* (2014). Briefly, Zymo-spin IIC columns (Zymo Research Corporation, Irvine, CA) were filled with 60 flies after removing the DNA binding matrix and centrifuged twice at 9000 g for 5 min at 4°C to collect hemolymph. Circulating ILP2 levels were measured by enzyme-linked immunosorbent assay (ELISA) using the protocol described in Bai *et al.* (2012).

Oral-glucose tolerance test

The oral-glucose tolerance test (OGTT) assay was adapted from Barry and Thummel (2016) and Haselton and Fridell (2010). Six replicates per time point and genotype, each containing five flies, were fasted overnight on a 1% agar solution. After fasting, one set of six replicates per genotype was frozen (“fasted” time). Replicate groups were refed in vials containing 10% glucose/1% agar medium for 2 h. While one set of replicates was frozen (“fed” time), the remaining replicate groups were transferred back to the fasting medium and frozen after 2 or 4 h postprandial (“2 or 4 h re-fast” times). Frozen flies were then homogenized in 100 μL phosphate-buffered saline and glucose was measured using the protocol described in Eveland *et al.* (2016).

Metabolic and feeding assays

Total triacylglycerol, glycogen, and protein levels were measured using protocols described in Eveland *et al.* (2016) and Jumbo-Lucioni *et al.* (2010). Circulating trehalose was measured in hemolymph using the method described in Eveland *et al.* (2016) and Park *et al.* (2014).

Metabolic rate was measured as CO₂ production using a flow-through respirometry system (Qubit System Research, Kingston, Ontario, Canada) and the approach was described in Jumbo-Lucioni *et al.* (2010).

Food intake was assessed using the Capillary Feeder assay described in Eveland *et al.* (2016) and Ja *et al.* (2007).

Starvation resistance assay

Survival under starvation conditions was assessed as described in De Luca *et al.* (2010).

Western blotting

Fly heads or bodies were snap-frozen and stored at −20°C. Frozen tissues were then homogenized in standard lysis buffer. Equal amounts of protein were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto polyvinylidene difluoride membrane (Thermo Scientific, Rockford), blocked in 5% bovine serum albumin, and incubated with 1:1000 rabbit β -Actin (# 4967; Cell Signaling, Danvers, MA); 1:1000 rabbit anti-Phospho-*Drosophila* Akt (Ser505) (#4054; Cell Signaling) and rabbit anti-Akt (#9272; Cell Signaling); 1:1000 rabbit anti-Phospho-ERK/MAPK (#4377; Cell Signaling) and rabbit anti-ERK/MAPK (#4695; Cell Signaling). The primary antibodies were incubated overnight at 4°C. The secondary antibody was incubated with 1:5000 horseradish peroxidase-conjugated goat anti-rabbit or goat anti-mouse for 1 h at room temperature and the signal developed using an enhanced chemiluminescence detection kit (Merk Millipore, Billerica, MA).

H₂O₂ measurements

Ten brains per genotype were dissected from live flies in 20 mM N-ethyl maleimide, which is a cell permeable alkylating agent that prevents air-induced oxidation during fly dissection (Albrecht *et al.*, 2011). Brain H₂O₂ levels were quantified using the Fluorimetric Hydrogen Peroxide Assay Kit (#MAK165-IKT; Sigma-Aldrich) according to the manufacturer's instructions. Fluorescence (λ_{ex} = 540/ λ_{em} = 590 nm) was measured with a BioTek microplate reader (BioTek Instruments, Winooski, VT).

Statistical analyses

Cox regression was used to compare survival of *ilp2>Sdc-RNAi* and *ilp2>w1118* flies under starvation conditions using SAS 9.4 software (SAS Institute, Cary, NC). One-way analysis of covariance (with body weight used as covariate) was used to assess differences between experimental and control groups in resting metabolic rate. Data from the other experimental assays were analyzed by two-tailed Student's *t*-test.

Results

Knockdown of *Sdc* in IPCs perturbs circulating ILP2 levels and glucose metabolism

We first analyzed the effects of *Sdc* knockdown in the IPCs on the expression of *ilp2*, *ilp3*, and *ilp5* genes. Contrary to our prediction, we found no significant differences in transcription levels of any of the *ilp* genes between *ilp2>Sdc-RNAi* and *ilp2>w1118* flies (Fig. 1A). However, quantification of circulating ILP2 in extracted hemolymph revealed that IPCs-specific *Sdc* knockdown flies had significantly less (45%) circulating ILP2 levels than controls (Fig. 1B).

Genetic manipulation of ILP2 production alters the levels of trehalose (the major circulating carbohydrate in the fly) in the hemolymph (Grönke *et al.*, 2010; Haselton and Fridell, 2010). Therefore, we next measured circulating trehalose in our experimental and control flies. As expected, there was a significant increase (52%) in hemolymph trehalose in flies with *Sdc* knocked down in the IPCs relative to controls (Fig. 2A). Previous studies also reported that IPCs-ablated flies had higher levels of stored metabolites (glycogen and triacylglycerol) and were more resistant to starvation than control flies, likely due to the increased levels of nutrient reserves (Broughton *et al.*, 2005). Consistent with these observations, we found that not only body glycogen levels were significantly higher (66%) in *ilp2>Sdc-RNAi* flies (Fig. 2B), but they also lived significantly longer than *ilp2>w1118* flies under starvation conditions (Fig. 2C). In contrast, no changes in body triacylglycerol stores between groups were observed (Fig. 2B).

FIG. 1. IPCs-specific *Sdc* knockdown leads to significantly decreased circulating ILP2 protein levels. (A, B) Despite no decrease in *ilps* mRNA levels (A), circulating ILP2 levels were significantly lower in IPC-specific *Sdc* knockdown (*ilp2>Sdc-RNAi*) flies compared to controls (*ilp2>w1118*) (B). Gene expression levels were measured by qPCR using mRNA extracted from heads of males ($n=6$). All genes were normalized to *rp49*. Circulating ILP2 levels were measured by ELISA and values represent the means of four independent replicates. ** $p < 0.01$ relative to control. Error bars represent SE. ELISA, enzyme-linked immunosorbent assay; ILP, insulin-like peptide; IPC, insulin-producing cell; qPCR, quantitative polymerase chain reaction; SE, standard error.

FIG. 2. IPCs-specific *Sdc* knockdown increases circulating trehalose and stored glycogen, and provides survival advantage to starvation independent of changes on energy balance. **(A)** Values represent the mean of circulating trehalose levels in IPCs-specific *Sdc* knockdown (*ilp2>Sdc-RNAi*) and control (*ilp2>w1118*) flies ($n \geq 7$). **(B)** Levels of triacylglycerol (TAG, left) and glycogen (GLYC, right) in homogenates of decapitated flies ($n = 10$ independent replicates). Measurements were normalized to total protein levels. **(C)** Survival curves under water-only starvation conditions. Starvation survival times were significantly longer in *ilp2>Sdc-RNAi* males compared to controls [log-rank test, chi square statistic (χ^2) = 16.3, $p < 0.0001$]. **(D)** Values represent the average amount of food ingested by five housed flies from 10 independent replicates. **(E)** Values represent the least-square means of whole-body CO₂ production (VCO₂), an index of resting metabolic rate, adjusted for live body weight ($n = 10$ independent replicates). * $p < 0.05$ and **** $p < 0.0001$ relative to control. Error bars represent SE.

To confirm that the effects on glucose metabolism were due to differences in the levels of circulating ILP2 between *ilp2>Sdc-RNAi* and *ilp2>w1118* flies rather than alterations in energy balance, we measured food intake and resting metabolic rate. We found no differences in either food intake (Fig. 2D) or in metabolic activity (Fig. 2E) between genotypes.

Knockdown of *Sdc* in IPCs leads to reduced glucose tolerance

Given that *ilp2>Sdc-RNAi* flies had reduced circulating ILP2 levels, we performed an OGTT assay to assess whether they would take longer than *ilp2>w1118* flies to restore their glucose levels to normal following ingestion of a large amount of glucose. As shown in Figure 3A, whereas there was no significant distinction in body glucose levels between *ilp2>Sdc-RNAi* and control flies after an overnight fast (“fasted”), we found that *ilp2>Sdc-RNAi* flies had significantly higher glucose levels immediately following 2h of glucose feeding (“fed”). The difference in glucose levels between groups was still significant 2h after the flies were again fasted (“2h re-fast”); however, this difference disappeared after 4h fasting (“4h re-fast”) (Fig. 3A). Overall, these data suggested that *ilp2>Sdc-RNAi* flies took longer than controls to clear post-prandial glucose due to glucose intolerance.

ILP-mediated receptor activation in *Drosophila* triggers the activation of Akt through phosphorylation (Alfa and Kim, 2016), we therefore measured AKT phosphorylation levels at Ser-505 in decapitated flies at the time immediately following the “fed” state. We found that AKT phosphorylation was decreased by 71% in *ilp2>Sdc-RNAi* flies relative to control flies (Fig. 3B). This finding further corroborates that *ilp2>Sdc-RNAi* flies are glucose intolerant.

FIG. 3. IPCs-specific *Sdc* knockdown leads to glucose intolerance. **(A)** Oral glucose tolerance test data (see text for details). Values represent the means of glucose in homogenates of decapitated flies ($n = 10$). **(B)** Left panel: quantification of phosphorylated AKT from western blot assays following glucose feeding (“fed,” $n = 4$). Right panel: representative western blot images in *ilp2>w1118* and *ilp2>Sdc-RNAi* flies. β-actin was used as loading control. * $p < 0.05$ and ** $p < 0.01$ relative to control. Error bars represent SE. MW, molecular weight.

FIG. 4. IPCs-specific *Sdc* knockdown reduces levels of head *Glut1* gene expression, ERK phosphorylation, and brain reactive oxygen species. (A) Gene expression levels were measured by qPCR using mRNA isolated from heads of *ilp2>w1118* and *ilp2>Sdc-RNAi* flies ($n=6$). Transcript levels of each target gene were normalized to *rp49*. (B) *Left panel*: quantification of phosphorylated ERK from western blot assays ($n=4$). *Right panel*: representative western blots images in *ilp2>w1118* and *ilp2>Sdc-RNAi* flies. β -Actin was used as loading control. (C) Values represent the means of H_2O_2 in fly brains ($n=16$). In all panels, $*p<0.05$ and $**p<0.01$ relative to control. Error bars represent SE. ERK, extracellular signal-regulated kinase.

Knockdown of *Sdc* in IPCs reduces levels of head *Glut1* gene expression, ERK phosphorylation, and brain reactive oxygen species

In mammals, the glucose-stimulated pancreatic insulin secretion (GSIS) mechanism is essential in maintaining glucose homeostasis (MacDonald *et al.*, 2005) and reduced expression of β cell *Glucose transporter (Glut)* genes has been associated with loss of GSIS in rodents (Unger, 1991). As mentioned in the introduction, growing evidence suggest that adult flies secrete ILPs in response to dietary glucose (Haselton and Fridell, 2010; Barry and Thummel, 2016). Additionally, it has been shown that IPCs-specific knockdown of the *Glut1* gene has no effect on *ilp2* mRNA levels, but it results in a drastic reduction in circulating ILP2 relative to controls (Park *et al.*, 2014). Based on these observations, we next compared mRNA levels of *Glut1* in the heads of *ilp2>Sdc-RNAi* and *ilp2>w1118* flies. Notably, we found that *ilp2>Sdc-RNAi* had 45% less *Glut1* transcript levels compared to control animals (Fig. 4A).

Recent results of both *in vitro* and *in vivo* studies have demonstrated a role for glucose-induced ERK1/2 activation in mammalian GSIS (Longuet *et al.*, 2005; Rondas *et al.*, 2012; Bowe *et al.*, 2013; Niu *et al.*, 2016). In light of our previous work showing a link between *Drosophila* fat body *Sdc* and ERK phosphorylation (Eveland *et al.*, 2016), we sought to measure the phosphorylation status of ERK in the heads of *ilp2>Sdc-RNAi* and control flies. As predicted, we found that knockdown of *Sdc* in the IPCs led to a 75% decrease in ERK phosphorylation levels (Fig. 4B).

Reactive oxygen species (ROS), such as H_2O_2 , regulate several signaling pathways, including activation of ERK in several mammalian cell types (Guyton *et al.*, 1996). Additionally, emerging data suggest that the NADPH oxidase-mediated production of H_2O_2 is involved in insulin secretion (Morgan *et al.*, 2009; Jayaram and Kowluru, 2012). Therefore, we next quantified H_2O_2 levels in the brains of *ilp2>Sdc-RNAi* and *ilp2>w1118* flies and observed that *ilp2>Sdc-RNAi* had remarkably lower (46%) levels of H_2O_2 (Fig. 4C).

Discussion

Emerging data suggest that signaling by direct cell–matrix interactions plays a pivotal role in tissues involved in the control

of systemic metabolism, such as adipose tissue, liver, and pancreas (Kaido *et al.*, 2006; Williams *et al.*, 2015). To this end, we previously reported that flies with a mutation in the *Sdc* gene, which encodes a transmembrane receptor that binds to matrix components (Narita *et al.*, 2004), had significantly lower *ilp2* (one of *Drosophila insulin-like peptide* genes) transcript levels in their heads than co-isogenic controls (De Luca *et al.*, 2010). Here, we show that, contrary to the results in the global *Sdc* mutant, knockdown of *Sdc* specifically in the IPCs did not have an effect on the expression of *ilp* genes. However, these flies exhibited reduced levels of circulating ILP2 protein and significant glucose intolerance compared to control flies. In line with results from previous IPC ablation studies and genetic deletion of *ilp2*, flies with reduced *Sdc* in the IPCs also had increased circulating trehalose and stored glycogen, and were more resistant to starvation conditions. Furthermore, they displayed a significant reduction in *Glut1* gene expression in their heads. This latter result is of particular interest considering that *Glut1* is a regulator of insulin secretion in *D. melanogaster* (Park *et al.*, 2014).

There are a variety of cell autonomous and nonautonomous mechanisms that have been identified to independently regulate gene expression and release of the IPCs-derived ILPs (Alfa and Kim, 2016). For instance, the expression of *ilp3* and *ilp5* is regulated by nutrient availability (Ikeya *et al.*, 2002) and reduction in *ilp3* transcript levels leads to downregulation of *ilp2* and *ilp5* expression, suggesting the presence of regulatory synergy in expression among IPCs-produced ILPs (Grönke *et al.*, 2010). Additionally, ILP6, which is synthesized in the fat body in response to nutrient availability, plays a role in regulating *ilp* expression and ILP peptide secretion in the IPCs (Bai *et al.*, 2012). The hypomorphic *Sdc* mutation significantly altered energy metabolism in flies (De Luca *et al.*, 2010). It is, therefore, plausible that the effect on *ilp2* expression previously seen in the *Sdc* mutant flies is due to an indirect cell nonautonomous signaling mechanism activated by the perturbed energy homeostasis.

Unlike previous work reporting an increase in fat storage in adult IPC knockdown flies compared to control flies (Haselton *et al.*, 2010), we did not observe any change in triacylglycerol levels between *ilp2>Sdc-RNAi* and *ilp2>w1118* flies. This finding is not surprising. Indeed, while the effects of ILPs on *Drosophila* glucose homeostasis are well established, there are contradictory results in the literature concerning their effects

on fat storage. For example, mutations in several insulin signaling pathway members increase body fat in flies [reviewed in Ref. Teleman (2009)]. Yet, activation of insulin signaling in the fat body also leads to lipid accumulation (DiAngelo and Birnbaum, 2009). Furthermore, *in vitro* work of glucose fluxes using isotope tracers showed that insulin promotes a significant reduction in the incorporation of D-[U-¹⁴C]glucose into glycogen, but it does not affect lipid synthesis in *Drosophila* (Ceddia *et al.* 2003). No differences in triacylglycerol levels have been reported between flies with mutations in the single *ilp2*, *ilp3*, and *ilp5* genes and their control flies (Grönke *et al.*, 2010). Given that in this study we only measured circulating ILP2 protein, it is possible that reduction of *Sdc* transcript levels in the IPCs may alter only the release of certain ILPs.

One fascinating finding in this study is the reduced levels of H₂O₂ and ERK phosphorylation in the heads of IPCs-specific *Sdc* knockdown flies. Several studies have suggested a link between Erk1/2 activation and glucose-stimulated insulin release from mouse islets, which appears to be mediated via effects on cytoskeletal remodeling (Longuet *et al.*, 2005; Park *et al.*, 2006; Bowe *et al.*, 2013). Recently, Jayaram and Kowluru (2012) proposed a model in which glucose-induced activation of the small GTPase ADP-ribosylation factor 6 (Arf6) in β cells controls ERK1/2 activation, which in turn leads to activation of Rac1 and thereby assembly and functional activation of NADPH oxidase, an H₂O₂-generating enzyme. NADPH oxidase-generated ROS in turn facilitate cytoskeletal remodeling and insulin release (Jayaram and Kowluru, 2012). Our data indicate that this mechanism might also occur in *D. melanogaster* and that *Sdc* could be involved. This idea is also supported by the fact that mammalian SDC4 regulates the activity of Arf6 (Brooks *et al.*, 2012). However, one limitation of the present study is that both ERK phosphorylation and H₂O₂ levels were not assessed specifically in the IPCs. Thus, additional work is needed to exclude potential cell nonautonomous effects of IPC-specific *Sdc* knockdown on the ERK pathway and H₂O₂ production.

Conclusions

The results presented here are the first demonstration of a cell autonomous role for *Sdc* in insulin release from IPCs rather than in insulin production. Recent studies in mammals argue for an important role of SDC4 in pancreatic β cell function (Cheng *et al.*, 2012). Preserving the function of β cells is critical to the prevention of type 2 diabetes (Kahn *et al.*, 2014), a disease that continues to affect the population of the United States and the world at an alarming rate. Thus, further studies investigating the role of *Drosophila* *Sdc* in the IPCs may help elucidate the role of its mammalian counterpart in β cell function and survival.

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Disclosure Statement

No competing financial interests exist.

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