PLEIOTROPIC ACTIONS OF IL-1 SIGNALING ON β-CELL FUNCTION AND GLUCOSE HOMEOSTASIS.

by

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A B S T R A C T

Significant research efforts have been devoted to understanding the role of aberrant immune function in the pathophysiology of metabolic disease. In this context, the pro-inflammatory cytokine IL-1β has emerged as a pleotropic regulator of glucose homeostasis. The diabetogenic consequences of IL-1 signaling have been examined extensively, with persistent IL-1 signaling inducing β-cell apoptosis, secretory dysfunction, and resulting in the dysregulation of glucose homeostasis. In contrast to its cytotoxic effects, however, the mechanisms underlying the anti-diabetic actions of IL-1β are relatively unknown.

The present work challenges the widely-accepted view that the glucoregulatory effects of IL-1β are primarily diabetogenic. The studies presented here elucidate a novel role of IL-1 signaling in the maintenance glucose homeostasis. Furthermore, this thesis demonstrates that the insulinotropic effects IL-1β are duration-dependent, with anti-diabetic actions predominating under conditions of acute IL-1 signaling. The present studies also confirm the cytotoxic actions of persistent IL-1 signaling on β-cell viability and demonstrate that post-translational modifier SUMO1 abrogates these effects.

Findings of the current thesis shed light on the relatively understudied aspects of IL-1β, both by establishing the necessity of IL-1 signaling in the regulation of glucose homeostasis and by implicating IL-1β as a mediator of islet compensation. These studies provide insights into potential mechanisms and environments regulating the pleiotropic effects of this cytokine and suggest that with strategic targeting of this pathway, the modulation of IL-1 signaling may emerge as a highly effective therapy in the treatment of type 2 diabetes (240 words).

PREFACE

This thesis is an original work by Catherine Hajmrle.

Chapter 2

This chapter is adapted from work published as C. Hajmrle, N. Smith, A.F. Spigelman, X. Dai, L. Senior, A. Bautista, M. Ferdaoussi, and P.E. MacDonald, "Interleukin-1 signaling contributes to acute islet compensation," *JCI Insight*, volume 1, issue 4, e86055. C. Hajmrle was responsible for data collection, analysis, and manuscript composition. N. Smith, A.F. Spigelman, X. Dai, L. Senior, and A. Bautista assisted with data collection; in these instances, C. Hajmrle performed analyses. M. Ferdaoussi contributed to the discussion, reviewed, and edited manuscript. P.E. MacDonald was the supervisory authority and was involved in concept formation and edits to the manuscript.

Chapter 3

This chapter is adapted from work published as C. Hajmrle, M. Ferdaoussi, G. Plummer, A.F. Spigelman, K. Lai, J.E. Manning Fox, and P.E. MacDonald, "SUMOylation protects against IL-1β-induced apoptosis in INS-1 832/13 cells and human islets," *American Journal of Physiology Endocrinology and Metabolism,* volume 307, issue 8, E664–E673. C. Hajmrle was responsible for data collection, analysis, and manuscript composition. M. Ferdaoussi assisted with data collection, analysis, and edits to the manuscript. G. Plummer, A.F. Spigelman, K. Lai, and J.E. Manning Fox assisted with data collection; in these instances, C. Hajmrle performed analyses. P.E. MacDonald was the supervisory authority and was involved in concept formation and edits to the manuscript.

Chapter 4

C. Hajmrle, A. Bautista, and M. Ferdaoussi were responsible for data collection and analysis. K. Suzuki generated the TAT-SUMO1 peptide. P.E. MacDonald and M. Ferdaoussi were the supervisory authorities and were involved in concept formation.

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For Warren

without whom this book
would have been written one year earlier,
but with a lot less love.

• • •

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GLOSSARY OF TERMS

°C	degrees Celsius
$[Ca^{2+}]_i$	intracellular calcium concentration
µg	microgram
µl	microlitre
µm	micron
µmol	micromole
Ad	adenovirus
ADP	adenosine diphosphate
ANOVA	analysis of variance
ATF4	activating transcription factor 4
ATP	adenosine triphosphate
AU	arbitrary units
AUC	area under the curve
Bcl	B-cell lymphoma
Bip	binding immunoglobulin protein
BMI	body mass index
BSA	bovine serum albumin
C/EBP	CCAAT-enhancing-binding protein
Ca^{2+}	calcium
$CaCl_2$	calcium chloride
CC3	cleaved caspase 3
CHOP	C/EBP homologous protein
CO_2	carbon dioxide
CsCl	cesium chloride
CsOH	cesium hydroxide
DAPI	4',6-diamidino-2-phenylindole, dihydrochloride
DMEM	Dulbecco's modified Eagle's medium
DNA	deoxyribonucleic acid
EDTA	ethylene diamine tetraacetic acid
EGTA	ethylene glycol tetraacetic acid
ER	endoplasmic reticulum
F-actin	filamentous actin
FA	focal adhesion
FBS	fetal bovine serum
FLIP	FLICE-inhibitory protein
g	standard gravity
GFP	green fluorescent protein
GLP-1	glucagon-like peptide 1
GSCa	glucose-stimulated $[Ca^{2+}]_i$
h	hour
HbA_{1c}	glycated hemoglobin
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HFD	high fat diet
HIF1α	hypoxia-inducible factor 1α
HIV	human immunodeficiency virus
IBMX	3-isobutyl-1-methylxanthine
IgG	immunoglobulin G
IKK	IκB kinase
IL	interleukin
IL-1R	interleukin 1 receptor
IL-1Ra	IL-1R antagonist
IL-1RAP	IL-1R accessory protein
iNOS	inducible nitric oxide synthase
IRAK4	IL-1R associated kinase 4
IκBα	inhibitor of NFκB
JNK	c-Jun terminal NH_2 kinase
K^+	potassium
K_{ATP}	ATP-sensitive potassium channel
KCl	potassium chloride
kg	kilogram
KRB	Krebs-Ringer bicarbonate buffer
l	litre
L-NIL	L-N^6-(1-iminoethyl)lysine dihydrochloride
LPS	lipopolysaccharide

MAPK	mitogen activated protein kinase
mg	milligram
$MgCl_2$	magnesium chloride
min	minute
ml	millilitre
mm	millimetre
mmol	millimole
mRNA	messenger ribonucleic acid
mV	millivolts
MYD88	myeloid differentiation primary response 88
MΩ	mega ohms
NaCl	sodium chloride
NADPH	nicotinamide adenine dinucleotide phosphate
NaH_2PO_4	monosodium dihydrogen orthophosphate
$NaHCO_3$	sodium bicarbonate
NEMO	NFκB essential modulator
NFκB	nuclear factor κB
ng	nanogram
NH_4Cl	ammonium chloride
nm	nanometre
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PDTC	ammonium pyrrolidinedithiocarbamate
PERK	protein kinase ribonucleic acid-like ER kinase
pF	picofarad
pg	picogram
PIAS	protein inhibitor of activated STAT
PKC	protein kinase C
PPARɣ	peroxisome proliferator-activated receptor ɣ
PTK2	protein tyrosine kinase 2
PXN	paxillin
RPMI	Roswell Park Memorial Institute medium
RT-PCR	reverse-transcriptase PCR
s	second
SDS-PAGE	polyacrylamide gel electrophoresis
SEM	standard error of the mean
SENP	sentrin/SUMO-specific protease
siRNA	small interfering RNA
SNAP-25	synaptosomal-associated protein, 25 kDa
SNARE	N-ethylmaleimide-sensitive factor attachment receptor
STAT	signal transducer and activator of transcription
SUMO	small ubiquitin-like modifier
T1D	type 1 diabetes mellitus
T2D	type 2 diabetes mellitus
TAT	HIV-transactivator of transcription
TNFα	tumor necrosis factor α
TRAF6	TNF receptor associated factor 6
TUNEL	terminal deoxynucleotidyl transferase dUTP nick end labeling
UBC9	ubiquitin-conjugating enzyme 9
UPR	unfolded protein response
wt/vol	weight per volume

GLOSSARY OF GENES

ACTB actin beta; *human*

Actb actin beta; *rat*

Adm adrenomedullin; *rat*

Atf4 activating transcription factor 4; *rat*

Bip heat shock protein family A member 5; *rat*

BNIP3 Bcl-2 interacting protein 3; *human*

Bnip3 Bcl-2/adenovirus EB1 interacting protein 3; *rat*

Cfos FBJ osteosarcoma oncogene; *rat*

Chop DNA-damage inducible transcript 3; *rat*

CXCL1 CXC motif chemokine ligand 1; *human*

Cxcl1 chemokine (CXC motif) ligand 1; *rat*

CXCL10 CXC motif chemokine ligand 10; *human*

HIF1A hypoxia inducible factor 1 alpha; *human*

IL1A interleukin 1 alpha; *human*

IL1B interleukin 1 beta; *human*

Il1b interleukin 1 beta; *rat*

IL6 interleukin 6; *human*

IL8 interleukin 8; *human*

IL1R1 interleukin 1 receptor, type I; *human*

Il1r1 interleukin 1 receptor, type I; *mouse*

IL1RN interleukin 1 receptor antagonist; *human*

Il1rn interleukin 1 receptor antagonist; *mouse*

Nos2 nitric oxide synthase 2, inducible; *rat*

TNFA tumor necrosis factor, *human*

MANUSCRIPTS

Manuscripts included in the thesis:

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Hajmrle, C., Ferdaoussi, M., Plummer, G., Spigelman, A.F., Lai, K., Manning Fox, J.E., and MacDonald, P.E. (2014). SUMOylation protects against IL-1β-induced apoptosis in INS-1 832/13 cells and human islets. Am. J. Physiol. Endocrinol. Metab. *307*, E664-E673.

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Dai, X.Q., Manning Fox, J.E., Chikvashvili, D., Casimir, M., Plummer, G., Hajmrle, C., Spigelman, A.F., Kin, T., Singer-Lahat, D., Kang, Y., et al. (2012). The voltage-dependent potassium channel subunit Kv2.1 regulates insulin secretion from rodent and human islets independently of its electrical function. Diabetologia *55*, 1709–1720.

Dai, X.Q., Plummer, G., Casimir, M., Kang, Y., Hajmrle, C., Gaisano, H.Y., Manning Fox, J.E., and MacDonald, P.E. (2011). SUMOylation regulates insulin exocytosis downstream of secretory granule docking in rodents and humans. Diabetes *60*, 838–847.

CHAPTER 1.
Introduction

1.1 GLUCOSE HOMEOSTASIS

1.1.a Glucose homeostasis.

Complex homeostatic mechanisms have evolved to provide a stable supply of energy substrates to central and peripheral tissues. Of particular importance are the mechanisms regulating glucose homeostasis; while certain tissues utilize a range of nutrients as energy substrates, cerebral activity is entirely dependent on glucose as an energy source (Wortis and Goldfarb, 1940). Hypoglycemia, therefore, results in numerous neurological complications, including seizures, coma, and eventual death. Conversely, persistent elevations in blood glucose concentrations also result in detrimental outcomes, including hyperglycemia-associated cardiovascular dysfunction, neuropathy, and other microvascular complications (reviewed in Forbes and Cooper, 2013). Consequently, homeostatic mechanisms have evolved to maintain blood glucose concentrations of the human body within the narrow range of 4 to 8 mmol/l (Zhou et al., 2009).

Impaired homeostatic control of glucose underlies diabetes mellitus—a metabolic disease affecting an estimated 2.4 million Canadians (Canadian Diabetes Association, 2013a) and over 422 million people globally (World Health Organization, 2016). Although diabetic patients are susceptible to hypoglycemic episodes, hyperglycemia is the primary diagnostic marker of diabetes mellitus (defined in Canadian Diabetes Association, 2013b and summarized in Table 1). Consistent with the negative effects of hyperglycemia on peripheral tissues, diabetic patients are at greater risk of heart failure (Aguilar et al., 2010), limb amputation (Humphrey et al., 1994), and overall mortality (Lind et al., 2014; Tancredi et al., 2015). Fortunately, even modest reductions in blood glucose concentrations have profound effects on patient outcome, significantly reducing the incidence of several diabetes-associated complications (Diabetes Control Complications Trial Research Group, 1993; UK

Prospective Diabetes Study Group, 1998a; 1998b). Reducing hyperglycemia through the use of lifestyle modifications or pharmacological therapy is a primary objective of the medical community (Canadian Diabetes Association, 2013c). Yet despite these intensive efforts, all-cause hospitalizations (Schneider et al., 2016) and mortality (Lind et al., 2014; Tancredi et al., 2015) remain elevated in the diabetic population, emphasizing the shortage of safe and effective therapeutic options in the treatment of this disease.

1.1.b Overview of homeostatic glucose regulation.

Glucose homeostasis is achieved by balancing the ingestion, production, and release of glucose into the blood with its uptake and storage into peripheral tissues (reviewed in Cahill, 1971). The human pancreas consists of both exocrine and endocrine tissue, with endocrine clusters dispersed within the surrounding exocrine mass. Clusters of endocrine pancreas are known as the islets of Langerhans, named to reflect their identification in the late 19th century by Paul Langerhans (Tattersall, 2010). The glucoregulatory roles of the islets of Langerhans were described in 1909 by MacCallum, who observed the maintenance of normoglycemia in a canine animal model despite complete destruction of the exocrine pancreas. Glucose homeostasis was attributed to the remaining islet-like structures that were unaffected by pancreatic duct ligation. In 1912, these structures were identified as islets of Langerhans, confirming the role of the endocrine pancreas as a regulator of glucose homeostasis (Kirkbride, 1912).

Pancreatic islets are multi-cellular structures composed of distinct cellular populations (Lane, 1907). Of the known islet cell types, pancreatic α- and β-cells are the most abundant, together comprising ~90% of human islets, at ~30% and ~60%, respectively (Cabrera et al., 2006; Figure 1A). The α- and β-cells of the islet secrete distinct and opposing

hormones, namely glucagon (Baum et al., 1962) and insulin (Lacy, 1959), which regulate blood glucose concentrations under conditions of hypo- and hyperglycemia. Elevations in blood glucose levels trigger insulin release from pancreatic β-cells (Figure 1B). Insulin binding to insulin receptors induces intracellular uptake and storage of glucose into several peripheral tissues (reviewed in Saltiel and Kahn, 2001), returning blood glucose concentrations to homeostatic levels. Conversely, during periods of hypoglycemia, glucagon promotes endogenous glucose production and release from glycogen stores, thereby restoring euglycemia (reviewed in Dunning and Gerich, 2007).

Abnormal insulin secretory responses are characteristic of diabetic patients (Seltzer and Smith, 1959; Yalow and Berson, 1960; Figure 1C), are present early in diabetic pathogenesis (Seltzer et al., 1967; Weyer et al., 1999), and occur concomitantly with the progression from a healthy to diseased state (Stancáková et al., 2009). Induction of secretory dysfunction in healthy subjects induces post-prandial hyperglycemia (Calles-Escandon and Robbins, 1987). Conversely, exogenous supplementation of insulin, aimed at mimicking the insulin profiles of healthy subjects, improves glucose tolerance in diabetic patients (Bruce et al., 1988). In addition to impaired insulin secretory responses, certain diabetic patients are characterized by an insensitivity to insulin (Himsworth, 1936) secondary to reduced insulin receptor signaling. Although resistance to insulin may facilitate the development of hyperglycemia, β-cell secretory dysfunction, rather than insulin resistance, is widely accepted as the primary defect in diabetic pathophysiology (reviewed in Gerich, 2002; Kahn, 2003).

1.1.c Glucose-stimulated insulin section.

Insulin is synthesized in the pancreatic β-cells as preproinsulin, where it is proteolytically cleaved to proinsulin and folded in the endoplasmic reticulum (ER).

Proinsulin is then packaged into secretory vesicles where it is cleaved by endopeptidases to its mature form (reviewed in Dodson and Steiner, 1998). Prevailing glucose concentrations are rapidly coupled to insulin secretion through a cascade of intracellular events known as "the triggering pathway" (reviewed in Henquin, 2000; Rorsman and Braun, 2013 and summarized in Figure 2A). Briefly, extracellular and intracellular glucose concentrations are rapidly equilibrated across the β-cell membranes following transport through high-capacity glucose transporters, specifically Glut2 in rodents and GLUT1 and 3 in humans (De Vos et al., 1995). Following transport, glucose is phosphorylated to glucose-6-phosphate by glucokinase (reviewed in Van Schaftingen, 1994). Glucose phosphorylation permits its metabolism, which is coupled to insulin secretion through ATP-sensitive K^+ (K_{ATP}) channels. Under non-stimulatory glucose conditions, β-cell membranes are hyperpolarized due to efflux of K^+ through open K_{ATP} channels. Generation of ATP, and reduction in ADP secondary to glucose metabolism, results in closure of β-cell K_{ATP} channels (Ashcroft et al., 1984). Suppression of K^+ efflux depolarizes β-cell membranes, activates voltage-gated Ca^{2+} channels, and results in extracellular Ca^{2+} influx (Rorsman and Trube, 1986).

Elevations in intracellular Ca^{2+} trigger granule exocytosis through activation of N-ethylmaleimide-sensitive factor attachment receptor (SNARE) protein complexes (reviewed in Rorsman and Renström, 2003 and summarized in Figure 2B). Following transport, SNARE proteins present on β-cell plasma membranes interact with SNARE proteins present on insulin granules resulting in insulin granule docking. Docked granules undergo granular acidification, which primes granules for Ca^{2+}-mediated exocytosis. Increased intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) induces a conformational change within the SNARE protein complex, inducing granule fusion to the plasma membrane and insulin release.

Complex mechanisms coordinate the movement of secretory granules from their storage sites within the β-cell to their exocytotic sites on the plasma membrane. The cytoskeleton, notably filamentous actin (F-actin), is an important regulator of this process (reviewed in Kalwat and Thurmond, 2013 and summarized in Figure 2C). In β-cells, a cortical web of F-actin is present below the plasma membrane functioning as a physical barrier between insulin granules and their exocytotic sites. Remodeling of the filamentous actin network subsequent to glucose metabolism allows granule mobilization to the plasma membrane, permitting insulin granules to be docked and subsequently primed for release.

Table 1. Diagnosis of diabetes mellitus.

diagnosis	HbA_{1c} (%)	plasma glucose (mmol/l)		
		fasting	OGTT	random
diabetes	≥ 6.5	≥ 7.0	≥ 11.1	≥ 11.1
pre-diabetes	6.0 – 6.4	--	--	--
IFG	--	6.1 – 6.9	--	--
IGT	--	--	7.8 – 11.0	--

Criteria for diagnosis of diabetes mellitus in Canadian patients (Committee et al., 2013). Plasma glucose concentrations following an overnight fast (fasting), 2 hours following a bolus administration of 75g of glucose (OGTT), or following a random assessment (random) are used to diagnose a patient as diabetic, pre-diabetic, or to indicate the patient has IFG or IGT. HbA_{1c}: glycated hemoglobin as a percentage of total hemoglobin. OGTT: oral glucose tolerance test. IFG: impaired fasting glucose. IGT: impaired glucose tolerance.

Figure 1. Pancreatic islets and insulin release. ►

A: Pancreatic section (5 μm) containing a human islet of Langerhans immunoreactive for insulin (green), glucagon (red), and nuclei (blue). **B:** Perifusion profile of insulin secretion in response to glucose (1.0 or 16.7 mmol/l, as indicated) from 15 isolated human islets from a single human donor. Insulin secretion was assessed as described in Section 3.3a and 3.3c, unless otherwise indicated. **C:** Insulin secretion normalized to ng of insulin released per islet from non-diabetic and type 2 diabetic human donors stimulated with glucose, as indicated (*n*=18, 20, 17, 21 from 6 and 7 donors, respectively). Insulin secretion was assessed as described in Section 2.3.a and 2.3.b, unless otherwise indicated. Data are mean ± SEM. ND: non-diabetic. T2D: type 2 diabetic.

Figure 1.

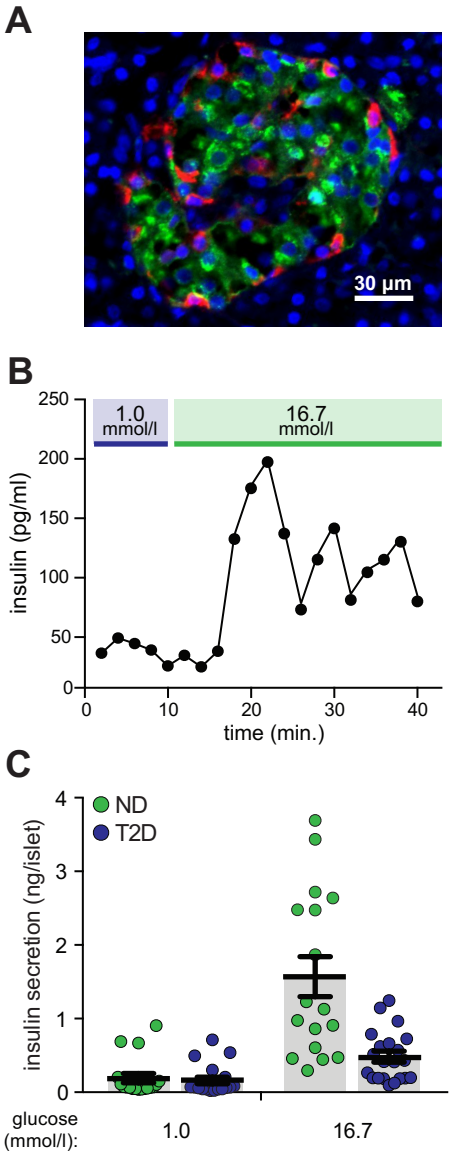
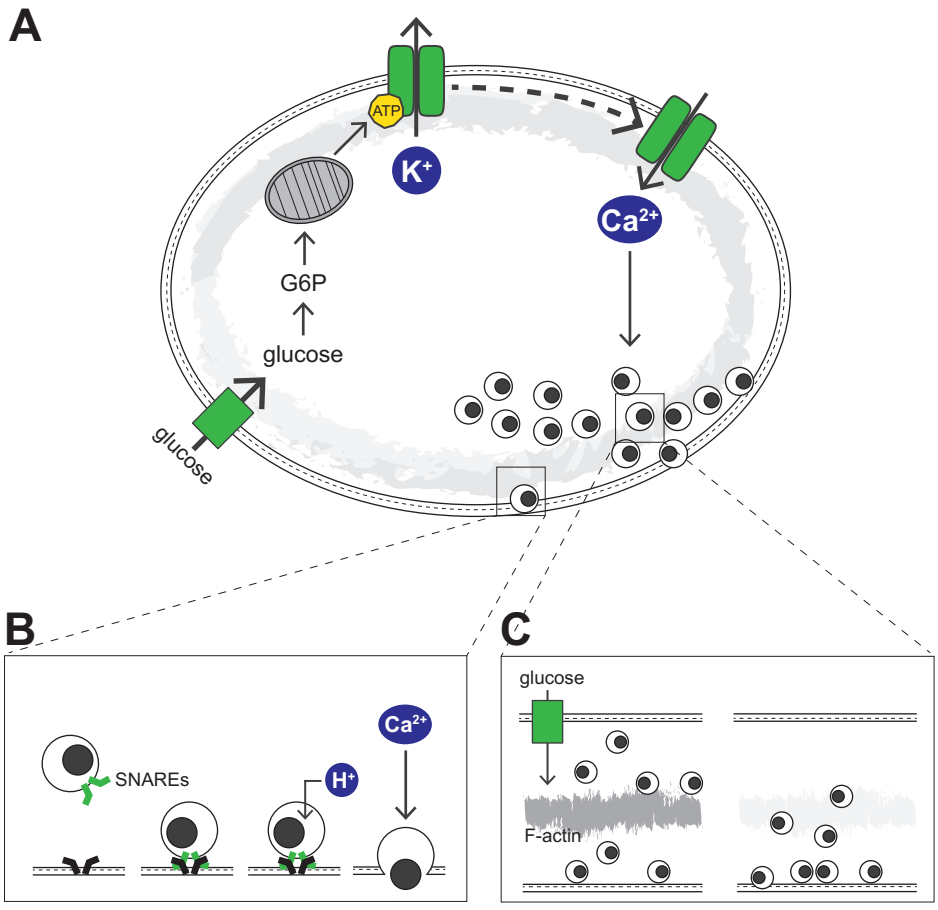

A
30 µm
B
1.0
mmol/l
16.7
mmol/l
insulin (pg/ml)
250
200
150
100
50
0
0
10
20
30
40
time (min.)
C
ND
T2D
insulin secretion (ng/islet)
4
3
2
1
0
glucose
(mmol/l):
1.0
16.7

Figure 2. Glucose-stimulated insulin secretion. ►

A: Glucose, transported into the β-cell through glucose transporters, is phosphorylated to glucose-6-phosphate (G6P). Mitochondrial metabolism of G6P enhances the ATP to ADP ratio, resulting in closure of K_{ATP} channels, membrane depolarization, and activation of voltage-gated Ca^{2+} channels. **B:** Insulin granules are recruited to the plasma membrane, where they are docked subsequent to the interaction of granule and membrane SNARE proteins. Granules undergo acidification, priming them for Ca^{2+}-stimulated granule release. **C:** Glucose-induced cortical F-actin remodeling allows granules access to secretory sites on β-cell plasma membranes.

Figure 2.


A
ATP
K+
Ca2+
G6P
glucose
glucose
B
SNAREs
H+
Ca2+
C
glucose
F-actin

1.2 PATHOPHYSIOLOGY OF TYPE 2 DIABETES

1.2.a Type 2 diabetes.

Although all diabetic patients are characterized by hyperglycemia, the cause of this metabolic imbalance varies amongst patients. Accordingly, patients are classified by the underlying etiology of their hyperglycemia (Canadian Diabetes Association, 2013b). Hyperglycemia arises in certain patients following an autoimmune-mediated destruction of the pancreatic β-cells (reviewed in Achenbach et al., 2005). These patients, classified as type 1 diabetic (T1D), are dependent on exogenous insulin therapy. In contrast, type 2 diabetes (T2D) is characterized by varying degrees of β-cell dysfunction in the presence of insulin resistance and is estimated to account for over 85% of diabetic cases (Forbes and Cooper, 2013). As such, significant focus is placed on identifying and consequently resolving the trigger of T2D. These efforts are complicated by the significant genetic and phenotypic heterogeneity of T2D patients and by the myriad of biochemical and physiological abnormalities characteristic of these patients. Indeed, over 66 susceptibility loci (Morris et al., 2012) and numerous environmental factors (World Health Organization, 2016) are currently associated with this disease, indicating that T2D is not initiated by one individual factor, but rather, arises in genetically susceptible individuals following exposure to permissive environmental conditions.

One such condition is overnutrition. Indicators of overnutrition, including elevated body mass index (BMI) (Chan et al., 1994; Colditz et al., 1990), increased waist-to-hip ratio (Ohlson et al., 1985), elevated plasma non-esterified fatty acid concentrations (Charles et al., 1997; Paolisso et al., 1995), and increased adipocyte size (Paolisso et al., 1995) are consistently identified as T2D risk markers in large prospective studies and across multiple cohort populations. Furthermore, several animal models of nutrient excess, including the

Zucker diabetic fatty rat (Lee et al., 1994), the ob/ob mouse (Mayer et al., 1951), and high-fat diet (HFD) fed mice (Mosser et al., 2015), are characterized by elevated glycemia and impaired glucose homeostasis. While obesity is regarded as a potent environmental risk factor for the development of T2D, not all obese patients become glucose intolerant, indicating that impaired *adaptation* to nutrient excess, rather than overnutrition itself, may underlie the progression to a diabetic state.

1.2.b β-cell compensation.

Nutrient excess induces insulin hypersecretion from pancreatic β-cells. This occurs acutely, via direct stimulation of β-cells by nutrients, and chronically, as a consequence of obesity-induced insulin resistance. The hypersecretion of insulin subsequent to increased insulin demand, known as β-cell compensation, restores normoglycemia that is disrupted by persistent nutrient excess, the presence of insulin resistance, or a combination of both (Kahn et al., 1993). β-cell compensation is mediated by several structural and functional modifications that either increase β-cell mass or enhance β-cell function, ultimately resulting in increased secretory capacity (reviewed in Prentki and Nolan, 2006 and summarized in Figure 3).

Post-mortem β-cell mass is increased in obese, non-diabetic individuals compared to lean controls (Butler et al., 2003). This consistent with animal models, where β-cell mass expansion is observed in rodents fed a HFD (Gonzalez et al., 2013; Mosser et al., 2015; Terauchi et al., 2007), in animals with genetically-induced insulin resistance (Kido et al., 2000), or in obese rodents (Liu et al., 2002). That glucose homeostasis is perturbed in certain models, despite expanded β-cell mass, supports a role for enhanced β-cell function in compensation. Indeed, the secretory function of individual rodent β-cells is enhanced during

compensation (Do et al., 2016; Gonzalez et al., 2013) and has been reported to occur secondary to increased glucose uptake and oxidation (Liu et al., 2002), enhanced sensitivity to insulinotropic stimuli (Nolan et al., 2006), up-regulation of insulin biosynthesis (Gonzalez et al., 2013), and an increase in granule fusion dynamics (Do et al., 2016). Due to various technical limitations, few studies investigating β-cell compensation in human subjects or in human donor tissue have been performed. Consequently, and in spite of the crucial role in the maintenance of a non-diabetic state, the regulators, mechanisms, and consequences of human β-cell compensation remain relatively unidentified and poorly understood.

1.2.c β-cell failure in type 2 diabetes.

The hyperglycemia that is characteristic of T2D patients develops when peripheral insulin demand exceeds β-cell secretory capacity (Ohn et al., 2016). The inability of β-cells to secrete sufficient amounts of insulin, referred to as β-cell failure, is a crucial component of T2D pathophysiology (reviewed in Prentki and Nolan, 2006 and summarized in Figure 3). Although the relative contribution remains a matter of debate (Kahn et al., 2009), both β-cell dysfunction and reduced β-cell mass contribute to type 2 diabetic β-cell failure.

1.2.c.i Reduced β-cell mass.

β-cell mass is maintained by the dynamic equilibrium of β-cell formation balanced with β-cell loss (Bonner-Weir, 2000). In T2D, β-cell mass is reduced (Butler et al., 2003; Rahier et al., 2008). That this results from an increase in β-cell loss, rather than a reduction in β-cell formation, is evidenced by increased apoptotic markers in β-cells of T2D donor pancreata (Butler et al., 2003) and isolated islets (Marchetti et al., 2007; 2004) compared to non-diabetic controls. These findings are paralleled in the well-established model of β-cell

compensation, the Zucker diabetic fatty rat, which is characterized by increased β-cell apoptosis and a 50% reduction in β-cell mass compared to obese, non-diabetic Zucker fatty rats (Pick et al., 1998). Together, these studies have provided incentive to examine the triggers of β-cell apoptosis in the context of type 2 diabetes, and in particular, in developing novel methods of reducing β-cell death.

1.2.c.ii Reduced β-cell function.

Although β-cells from T2D donors have reduced glucose-stimulated insulin release compared to non-diabetic controls (Del Guerra et al., 2005; Ferdaoussi et al., 2015; Lupi et al., 2008; Marchetti et al., 2007; 2004), the dysfunction is surmountable (Del Guerra et al., 2005; Ferdaoussi et al., 2015; Marchetti et al., 2004). Indeed, that re-introduction of certain co-factors and peptides restores β-cell function indicates the presence of functional triggering pathway machinery in these cells (Ferdaoussi et al., 2015). This is supported by findings that response to amino acid- and K_{ATP}-inhibition-induced insulin secretion is relatively conserved T2D islets (Del Guerra et al., 2005). The reduced sensitivity of T2D β-cells to insulinotropic stimuli is not limited to glucose, as the stimulatory effects of glucagon-like peptide 1 (GLP-1) on glucose-stimulated insulin secretion are also reduced in T2D islets (Lupi et al., 2008). Additional insulinotropic stimuli to which T2D β-cells are insensitive, and the mechanism(s) of action underlying this dysfunction, have yet to be fully elucidated.

1.2.c.iii Mechanisms of β-cell failure.

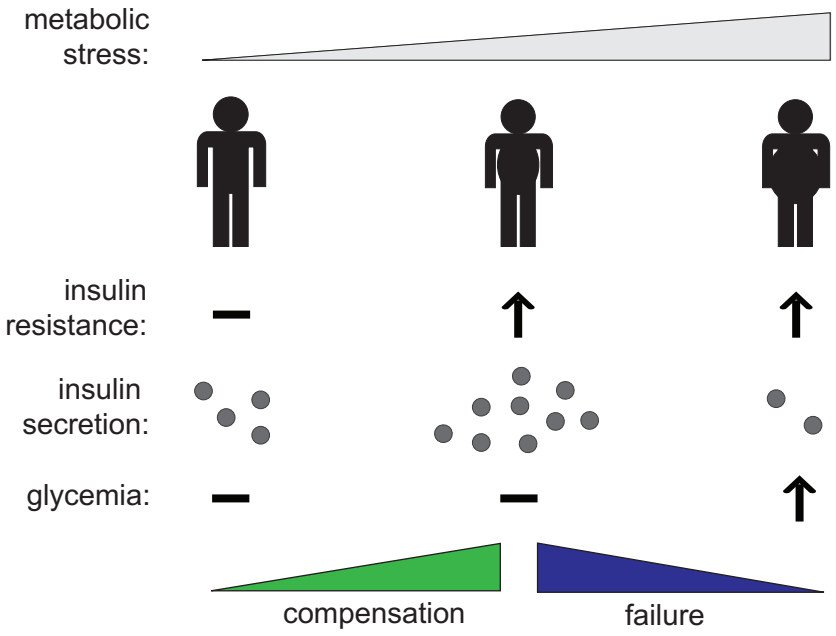
In addition to inducing hyperglycemia, the inability of T2D β-cells to meet peripheral insulin demand also results in hyperlipidemia (reviewed in Saltiel and Kahn, 2001). Death and dysfunction induced by hyperglycemia and hyperlipidemia are termed glucotoxicity (Maedler et al., 2002) and lipotoxicity (Lupi et al., 2002), respectively, reflecting their potent

cytotoxic effects on pancreatic β-cells. The detrimental actions of high glucose and elevated free fatty acids are mediated in part through induction of ER stress (reviewed in Cnop et al., 2005). While upstream mechanisms differ, hyperglycemia (Maedler et al., 2002), hyperlipidemia (Böni-Schnetzler et al., 2009; Igoillo-Esteve et al., 2010), and ER stress (Brozzi et al., 2015) all up-regulate intra-islet inflammation—a potent inducer of β-cell death and dysfunction (Section 1.3.b). The association between several β-cell death pathways and inflammation has highlighted the crucial role of the innate immune response in T2D pathogenesis. Indeed, T2D is considered an inflammatory disease (Donath and Shoelson, 2011), with the efficacy of anti-inflammatory strategies in the treatment of T2D being examined in several clinical studies (Donath, 2016).

Figure 3. β-cell compensation and maladaptation. ►

Insulin is released in response to hyperglycemia and reduces blood glucose concentrations secondary to insulin-induced uptake of glucose into peripheral tissues. Demand for insulin is increased upon exposure to nutrient excess, the development of insulin resistance, or a combination of both. To maintain normoglycemia, pancreatic β-cells enhance insulin secretion in a process known as β-cell compensation. Over time in susceptible individuals, β-cells are no longer able to provide sufficient amounts of insulin to overcome increased demand and hyperglycemia develops in a process known as β-cell failure.

Figure 3.


metabolic stress:
insulin resistance:
insulin secretion:
glycemia:
compensation
failure

1.3 TYPE 2 DIABETES AS AN INFLAMMATORY DISEASE

1.3.a Innate immune response.

The primary function of the immune system is to protect, resolve, and recover from injurious stimuli (reviewed in Male, 2013). There are two branches of the immune system: the adaptive immune response, which depends on specific pathogen recognition and repeated pathogen exposure, and the innate immune response, which is activated, in a relatively unspecific manner, by pathogen invasion, tissue damage, or an abnormal internal environment. Inflammation is a critical component of the innate immune system and is involved in immune cell recruitment to the site of injury. Inflammation-induced recruitment of immune cells, including phagocytotic macrophages, is mediated by up-regulation of pro-inflammatory cytokines. That nutrient excess induces an inflammatory response (reviewed in Wellen and Hotamisligil, 2005), suggests that overnutrition is perceived by the body as a threat requiring resolution. As such, nutrient excess is often referred to as metabolic stress, particularly in the context of immunometabolism.

1.3.b Type 2 diabetes and inflammation.

The role of the innate immune system as a mediator of T2D pathophysiology emerged in the late 1990s (Pickup and Crook, 1998) following the observation that the inflammation-associated proteins amyloid A, C-reactive protein, and the pro-inflammatory cytokine interleukin (IL)-6, were elevated in T2D patients (Pickup et al., 1997). The serum profiles of these patients were indicative of mild inflammation, consistent with systemic activation of the innate immune response. Elevation in inflammatory markers and cytokines were subsequently found to predict diabetes risk in several prospective cohort studies

(Pradhan et al., 2001; Schmidt et al., 1999; Spranger et al., 2003). A causal role of inflammation in diabetic pathophysiology is evidenced by the ability of anti-inflammatory therapies to improve glucose homeostasis in T2D patients (Goldfine et al., 2008; Hundal et al., 2002; Koska et al., 2009; Reid et al., 1957). Although a well-established regulator of insulin resistance (reviewed in Shoelson et al., 2006), inflammation is now recognized as a potent inducer of β-cell dysfunction. Inflammatory markers and immune cell infiltrates are present in islets of T2D patients (Böni-Schnetzler et al., 2008; Ehses et al., 2007; Igoillo-Esteve et al., 2010; Maedler et al., 2002; Richardson et al., 2009) and in several animal models of T2D (Ehses et al., 2007; Homo-Delarche et al., 2006; Weksler-Zangen et al., 2008). Indeed, inhibition of inflammatory signaling reduces metabolic-stress induced cytotoxicity in isolated human β-cells (Maedler et al., 2002; Zeender et al., 2004) and enhances post-prandial plasma insulin concentrations in T2D patients (Goldfine et al., 2008).

1.3.c IL-1β as a master regulator of inflammation.

The ability of the pro-inflammatory cytokine IL-1β to induce production of several inflammation-associated proteins indicates that IL-1β is a master regulation of inflammation (reviewed in Dinarello, 2011). This is evidenced most convincingly in patients with mutations in the gene encoding the IL-1 receptor antagonist, *IL1RN*, which results in increased IL-1 signaling. These patients are characterized by robust activation of the acute phase response that is rapidly resolved following exogenous administration of IL-1 receptor antagonist (Aksentijevich et al., 2009; Reddy et al., 2009). Indeed, pharmacological antagonism of IL-1 activity in human subjects reduces systemic expression of the pro-inflammatory markers IL-6 and C-reactive protein (Larsen et al., 2007; Ridker et al., 2012;

Sloan-Lancaster et al., 2013). IL-1β is a proximal regulator of inflammation in human pancreatic islets, up-regulating expression of IL-8, IL-6 (Böni-Schnetzler et al., 2008), and IL-15 (Brozzi et al., 2015). Furthermore, human β-cell chemokine and cytokine expression is positively correlated with *IL1B* expression, where inhibition of IL-1 signaling reduces cytokine and palmitate-induced up-regulation of the inflammation-associated genes *IL6, CXCL1, IL8, IL1B,* and *TNFA* (Igoillo-Esteve et al., 2010).

1.4 INTERLEUKIN-1B

1.4.a Interleukin-1β.

IL-1β is a pro-inflammatory cytokine belonging to the IL-1 cytokine family. In addition to its immunological roles, IL-1β is a central regulator of metabolism, translating metabolic stress into inflammation and mediating inflammation-induced metabolic dysfunction (reviewed in Donath et al., 2013). In healthy individuals, plasma concentrations of IL-1β are generally within the range of 0.1 to 5.0 pg/ml (Alexandraki et al., 2008; Bissonnette et al., 2015; El-Wakkad et al., 2013; Misaki et al., 2010; Spranger et al., 2003). During acute conditions of inflammatory stress, such as those associated with severe trauma or septic shock, plasma IL-1β concentrations reach upwards of 100 pg/ml (Gentile et al., 2013). IL-1β concentrations are also elevated in overweight subjects (El-Wakkad et al., 2013; Misaki et al., 2010), with increases in IL-1β ranging from 1.5- to 15-fold over lean controls.

1.4.a.i Production and release of IL-1.

The interleukin-1 family encompasses the classical IL-1 cytokines IL-1α, IL-1β, and the IL-1 receptor antagonist (IL-1Ra) (Garlanda et al., 2013). Two distinct genes, namely *IL1A* and *IL1B*, encode the two functional IL-1 isoforms, IL-1α and IL-1β, respectively (March et al., 1985). IL-1α is expressed constitutively in several cell types and is active in its precursor form. It is retained in the cytosol and is released from cells secondary to cellular necrosis. Once IL-1α is released, it binds to IL-1R of neighboring cells activating IL-1 signaling pathways (reviewed in Garlanda et al., 2013). In contrast, transcription of *IL1B* requires stimulation by factors, including microbial products such as lipopolysaccharide (LPS) or cytokines such as IL-1β (reviewed in Dinarello, 2011).

Following stimulation, *IL1B* is transcribed as an inactive precursor (March et al., 1985) requiring proteolytic cleavage to become active. Numerous proteases capable of cleaving pro-IL-1β have been identified (Netea et al., 2015), with certain proteases, namely caspase 1, requiring post-translational activation by an intracellular protein complex termed the inflammasome (Martinon et al., 2002). Although the post-translational processing of IL-1β represents a crucial regulatory step for the induction of IL-1β activity, it is important to note that several of the currently identified IL-1β proteases, including the protease caspase 1, are not specific to IL-1β (reviewed in Netea et al., 2015).

IL-1β is released from a variety of cells including the pancreatic islets (Arnush et al., 1998). While the ability of pancreatic β-cells to secrete IL-1β has been reported (Böni-Schnetzler et al., 2008; Maedler et al., 2002), others have attributed intra-islet production of IL-1β to resident islet lymphoid (Arnush et al., 1998) or ductal (Welsh et al., 2005) cells.

1.4.a.ii IL-1 receptor activation and intracellular signaling.

Biological actions of IL-1α and IL-1β are mediated through activation of the IL-1 receptor type 1 (IL-1R1; reviewed in Sims and Smith, 2010). Briefly, binding of IL-1R1 induces its heterodimerization with the IL-1 receptor accessory protein (IL-1RAP). Dimerization facilitates recruitment and activation of several downstream signaling molecules, including myeloid differentiation primary response 88 (MYD88), IL-1R associated kinase 4 (IRAK4), and tumor necrosis factor receptor associated factor 6 (TRAF6) (reviewed in Akira and Takeda, 2004; Arthur and Ley, 2013). Cellular specificity of IL-1 action is mediated by diverse post-receptor mechanisms; pathways pertinent to pancreatic β-cell function are discussed in Section 1.4.b. IL-1R1 activation is inhibited by the endogenously expressed IL-1Ra (Hannum et al., 1990). While IL-1Ra binds to IL-1R1, it is

unable to induce the recruitment of IL-1RAP (Greenfeder et al., 1995), thereby competitively antagonizing IL-1R1 activation by IL-1α and IL-1β.

1.4.b Mechanisms of IL-1β-induced β-cell cytotoxicity.

IL-1β protein and transcript expression are increased in islets of T2D donors (Böni-Schnetzler et al., 2008; Maedler et al., 2002), in animal models of T2D (Ehses et al., 2009; Maedler et al., 2002; Westwell-Roper et al., 2015), and are up-regulated following exposure of non-diabetic human islets to cytokines (Arnush et al., 1998) or metabolic stress (Böni-Schnetzler et al., 2009; Igoillo-Esteve et al., 2010; Maedler et al., 2002). β-cell death and dysfunction are induced by the prolonged exposure of human (Rabinovitch et al., 1990) and rodent (Mandrup-Poulsen et al., 1986) islets to IL-1β. Conversely, antagonism of IL-1 signaling reduces human β-cell cytotoxicity induced by inflammatory (Arnush et al., 1998) or metabolic (Maedler et al., 2002) stress and improves glycemia in animal models of T2D (Ehses et al., 2009; Westwell-Roper et al., 2015).

Despite the well-established role of β-cell death in T2D pathogenesis, apoptotic pathways of the human pancreatic β-cell remain relatively uncharacterized (Thomas and Biden, 2009). Nuclear factor κB (NFκB) and c-Jun terminal NH_2 kinase (JNK) have emerged as mediators of IL-1β-induced human β-cell cytotoxicity. While IL-1β-induced activation of inducible nitric oxide synthase (iNOS) and ER stress are also proposed regulators of IL-1β-mediated cell death (Cnop et al., 2005), evidence of these pathways in IL-1β-induced β-cell dysfunction is obtained from studies using cytokine cocktails. This experimental design is chosen in response to reports demonstrating an inability of IL-1β alone to induce human β-cell dysfunction (Eizirik et al., 1993) or rodent β-cell apoptosis (Cardozo et al., 2005). While the sufficiency of IL-1β alone to induce β-cell cytotoxicity is debated, that IL-1β exerts

detrimental effects on β-cell function when it is present in combination with other cytokines is well-accepted. In support of the ability of IL-1β to induce ER stress alone, however, are findings that IL-1β impairs intracellular Ca^{2+} handling in human (Figure 4) and mouse islets (Dula et al., 2010) in a manner comparable to aberrant $[Ca^{2+}]_i$ handling induced by pharmacological inducers of ER stress (Dula et al., 2010; O'Neill et al., 2013). Although iNOS and ER stress are proposed mediators of IL-1β-induced dysfunction, the ability of IL-1β alone to activate iNOS or ER stress remains unknown.

1.4.b.i Nuclear factor κB.

The mechanisms regulating the activation of NFκB are classified into canonical (reviewed in Hayden and Ghosh, 2008 and summarized in Figure 5) or non-canonical (reviewed in Sun, 2012) pathways. Under non-stimulatory conditions, NFκB is sequestered in the cytosol, thereby restricting NFκB-mediated gene transcription. In the canonical pathway, the inhibitor of NFκB (IκBα) mediates cytosolic sequestration of NFκB. Activation of IL-1R1 induces a series of intracellular events resulting in the phosphorylation of IκBα, marking it for ubiquitylation and subsequent proteosomal degradation. Phosphorylation of IκBα is mediated by the IκB kinase (IKK) complex, which is composed of NFκB essential modulator (NEMO), IKKβ, and IKKα. Once liberated from IκBα, canonical NFκB (comprised of p50 and RelA/p65 subunits) translocates to the nucleus and activates transcription of target genes.

IL-1β induces activation of NFκB in human islets (Flodström et al., 1996; Maedler et al., 2002). Inhibition of NFκB activity, either pharmacologically (Maedler et al., 2002) or via up-regulation of a non-degradable form of IκBα (Giannoukakis et al., 2000), restores secretory function and survival in IL-1β-treated human β-cells. Consistent with its

detrimental role in vitro, constitutive activation of NFκB in murine pancreatic β-cells results in severe hyperglycemia and hypoinsulinemia secondary to substantial β-cell loss (Salem et al., 2014). Human β-cell cytotoxicity induced by IL-1β-mediated activation of NFκB is attributed to the NFκB-induced up-regulation of Fas ligand expression (Maedler et al., 2002). Fas ligand binding to neighboring β-cell Fas receptors activates caspase 8, resulting in pancreatic β-cell death. NFκB also regulates *IL1B* transcription (Böni-Schnetzler et al., 2008) and may indirectly amplify β-cell death through the feed-forward induction of IL-1β-induced apoptosis.

1.4.b.iii Inducible nitric oxide synthase.

Inducible nitric oxide synthase belongs to the nitric oxide synthase family of enzymes that catalyze the production of nitric oxide (Förstermann and Sessa, 2012). β-cell transcription of *Nos2* is regulated by cytokine-induced activation of the nuclear transcription factor NFκB (Cardozo et al., 2001). The detrimental effects of iNOS on rodent β-cell function are secondary to nitric oxide production (Southern et al., 1990), which induces β-cell death through a nitric oxide-induced up-regulation of the ER stress mediators *Chop* and *Atf4* (Cardozo et al., 2005).

In contrast to rodent islets, iNOS appears to have a negligible role in cytokine-induced human β-cell cytotoxicity. It is not required for IL-1β- (Maedler et al., 2002) or cytokine- (Eizirik et al., 1994; Rabinovitch et al., 1994) induced human β-cell death. Indeed, inhibition of iNOS is unable to reduce cytokine-mediated ER stress in human islets (Brozzi et al., 2015). Nevertheless, the role of iNOS in humans should not be discounted, as exogenous administration of nitric oxide donors potentiates activation of pro-apoptotic JNK (Størling et al., 2005) and cell death (Eizirik et al., 1996a) in human islets. Furthermore, given the

relative ease of quantifying nitric oxide generation and iNOS expression in β-cells, measurements of these parameters are used occasionally as surrogate markers of NFκB activity.

1.4.c Confounding effects of IL-1β.

In contrast to in vitro evidence demonstrating a cytotoxic role of IL-1β on β-cell survival and function, elevated levels of IL-β alone are not associated with increased T2D risk (Spranger et al., 2003). Rather, reports from three large prospective cohort studies identify elevated IL-1Ra concentrations as a T2D risk factor (Herder et al., 2009; Luotola et al., 2011; Salomaa et al., 2010), where an increase in IL-1Ra is observed as early as 13 years prior to the onset of diabetes (Carstensen et al., 2010). While this increase in IL-1Ra has been described as a "futile" attempt to counteract an up-regulation in IL-1β (Herder et al., 2015), IL-1β concentrations in these cohorts were not reported. Furthermore, sustained inhibition of IL-1 signaling, induced by a genetic up-regulation of IL-1Ra, had no effect on diabetes risk in a meta-analysis of over 80,000 individuals (Interleukin 1 Genetics Consortium, 2015).

Several clinical trials examining the effects of IL-1R1 antagonism on type 2 diabetic outcomes have been conducted (and are summarized in Table 2). These studies have produced conflicting results, with certain reports demonstrating an inability of IL-1R1 antagonism to significantly reduce glycemia (Ridker et al., 2012; Rissanen et al., 2012; van Asseldonk et al., 2011; van Poppel et al., 2014), despite effectively reducing systemic inflammation. Of the studies where improvements in glycemia were observed (Cavelti-Weder et al., 2012; Larsen et al., 2007; Sloan-Lancaster et al., 2013; Stahel et al., 2016), reductions in glycated hemoglobin (HbA_{1c})—a marker of long-term glycemic control—were modest, with the majority of studies reporting improvements less than 0.50% versus placebo

controls (Table 2). In comparison, currently available oral anti-diabetic agents reduce HbA_{1c} levels by 0.75 and 1.25% (Sherifali et al., 2010).

These in vivo findings are consistent with animal models of reduced IL-1 signaling, where mice deficient in *Il1r1* (García et al., 2006) and *Il1b* (Maedler et al., 2006) are glucose intolerant. Conversely, mice deficient in *Il1rn* are normoglycemic (Matsuki et al., 2003) and have normal islet morphology (Somm et al., 2006). Together, these findings suggest that regulation of glucose homeostasis by IL-1β may be pleiotropic in nature, with IL-1 signaling exerting both positive and negative effects on β-cell function in vivo. Indeed, insulinotropic effects of IL-1β are known (Section 1.4.d) and may underlie the discrepancies observed.

1.4.d Insulinotropic effects of IL-1β.

1.4.d.i Proliferation-dependent effects.

Long-term exposure of human β-cells to low doses of IL-1β increases β-cell proliferation. The proliferative actions of IL-1β are mediated by the IL-1β-induced up-regulation of FLICE-inhibitory protein (FLIP), where the genetic down-regulation of FLIP in β-cells inhibits IL-1β-induced β-cell proliferation (Maedler et al., 2006). Indeed, mice deficient in *Il1b* are characterized by reduced FLIP and insulin transcript expression that occurs concomitantly with reduced islet insulin content and impaired glucose-stimulated insulin secretion (Maedler et al., 2006).

1.4.d.ii Proliferation-independent effects.

IL-1β induces hypoglycemia in rodents (Besedovsky and del Rey, 1987; del Rey and Besedovsky, 1987; Oguri et al., 2002) and humans (Crown et al., 1991) and increases plasma insulin (Cornell, 1989; del Rey and Besedovsky, 1987; Ling et al., 1995) within 4 hours of

cytokine infusion. Although the mechanism is unknown, the rapid nature of the exposure indicates that the hypoglycemic effects of IL-1β are not secondary to enhanced proliferation. Rather, IL-1β acts on abundantly expressed IL-1R1 on pancreatic β-cells (Böni-Schnetzler et al., 2009; Eizirik et al., 1992) to potentiate stimulus-induced insulin secretion from perfused pancreata (Yelich, 1990; 1992; Zawalich and Zawalich, 1989), isolated islets (Arous et al., 2015; Borg and Eizirik, 1990; Comens et al., 1987; Eizirik and Sandler, 1989; Eizirik et al., 1992; 1993; 1995; Helqvist et al., 1989; Jeong et al., 2002; Palmer et al., 1989; Welsh et al., 1991; 1989; Zawalich and Diaz, 1986; Zawalich and Zawalich, 1989), and purified β-cells (Arous et al., 2015).

That the insulinotropic effects of IL-1β are stimulus-dependent indicates that IL-1β-induced potentiation of insulin secretion is not due to unregulated β-cell lysis. IL-1β is unable to enhance insulin secretion under non-stimulatory glucose conditions (Arous et al., 2015; Comens et al., 1987; Eizirik et al., 1995; Zawalich and Zawalich, 1989) and requires triggering pathway activation (Arous et al., 2015; Eizirik et al., 1995). Initially, the insulinotropic effects of IL-1β were attributed to its ability to increase glucose oxidation (Borg and Eizirik, 1990; Eizirik and Sandler, 1989; Eizirik et al., 1992). However, that IL-1β potentiates KCl- (Arous et al., 2015) and sulfonylurea- (Eizirik et al., 1995) induced insulin secretion suggests that its effects are downstream of glucose metabolism. An IL-1β-induced rise in intracellular Ca^{2+} concentrations has also been proposed as a mediator of its insulinotropic actions (Arous et al., 2015; Borg and Eizirik, 1990; McDaniel et al., 1988), although this has been disputed (Eizirik et al., 1995; Helqvist et al., 1989; Welsh et al., 1989). While IL-1β mediated potentiation of stimulus-induced insulin secretion requires extracellular Ca^{2+} (Eizirik and Sandler, 1989) and functional voltage-gated Ca^{2+} channels

(Eizirik et al., 1995), whether its insulinotropic actions are mediated through an elevation in intracellular Ca^{2+} remains unknown.

Recently, the acute insulinotropic actions of IL-1β were attributed to IL-1β-induced modulation of focal adhesions (FA) proteins (Arous et al., 2015). FA proteins function to integrate the extracellular matrix with cytoskeletal proteins and are known regulators of insulin secretion (reviewed in Arous and Halban, 2015). IL-1β increases FA length and activates the FA-associated proteins protein tyrosine kinase 2 (PTK2) and paxillin (PXN) (Arous et al., 2015). However, that IL-1β-mediated potentiation of insulin secretion persists in the presence of reduced focal adhesion length (Arous et al., 2015) suggests the presence of a FA-independent pathway.

As the insulinotropic effects of IL-1β have been investigated primarily in vitro, the physiological role of IL-1β-mediated potentiation of stimulus-induced insulin secretion remains unknown. *IL1B* is increased acutely by post-prandial hyperglycemia (Sage et al., 2012) and is up-regulated in human islets following a 4-day exposure to high glucose (Maedler et al., 2002) or elevated free fatty acids (Böni-Schnetzler et al., 2009). Expression of IL-1β downstream target genes are increased within 3 days of HFD feeding in mice (Lee et al., 2011), suggesting that acute insulinotropic actions of IL-1β play an important role in early responses to metabolic stress. Whether IL-1 signaling has a role in acute β-cell compensation, however, remains unknown.

Figure 4. IL-1β impairs human islet $[Ca^{2+}]_i$ responses to glucose. ►

Human islets were treated for 48 hours with 5 or 25 mmol/l glucose in the presence of either vehicle or IL-1β (10 ng/ml). $[Ca^{2+}]_i$ were assessed using methods described in Section 2.3.a and 2.3c, unless otherwise indicated. **A:** Representative responses of $[Ca^{2+}]_i$ in human islets stimulated with glucose (16.7 mmol; arrow). **B:** Fold increase in glucose-stimulated $[Ca^{2+}]_i$ (GSCa) responses (n=4, 4, 5, 4; from 3 donors). **C:** Baseline $[Ca^{2+}]_i$ measurements during low glucose (0.5 mmol/l) stimulation (n=4, 4, 5, 4; from 3 donors). n values correspond to data points from left to right, respectively. Data are mean ± SEM compared with 2-way ANOVA followed by Tukey post-test. *P<0.05 versus 5 mmol/l vehicle control.

Figure 4.

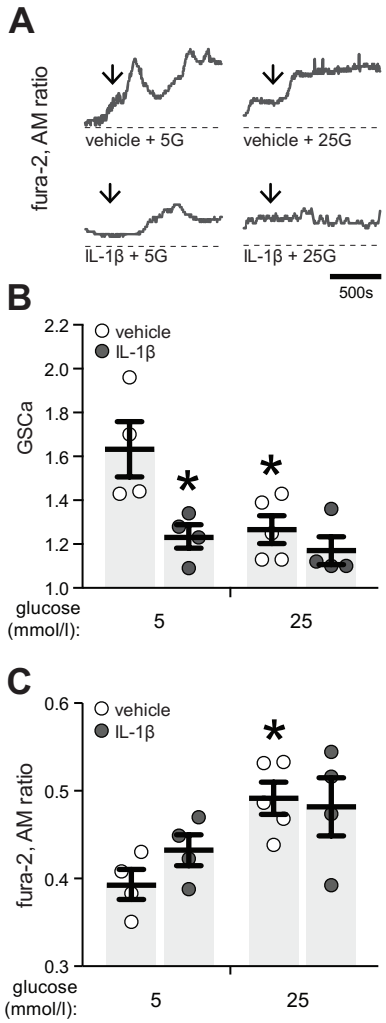

A
fura-2, AM ratio
vehicle + 5G
vehicle + 25G
IL-1β + 5G
IL-1β + 25G
500s
B
vehicle
IL-1β
GSCa
2.2
2.0
1.8
1.6
1.4
1.2
1.0
glucose (mmol/l):
5
25
C
vehicle
IL-1β
fura-2, AM ratio
0.6
0.5
0.4
0.3
glucose (mmol/l):
5
25

Figure 5. NFκB signaling pathway. ►

Binding of IL-1 to IL-1R1 induces IL-1R1 dimerization with IL-1RAP. Dimerization recruits MYD88, which in turn recruits IRAK proteins, ultimately resulting in the activation of TRAF6. Through a cascade of intracellular signaling events, the IKK complex is activated and phosphorylates IκBα. Phosphorylation of IκBα signals its ubiquitylation and subsequent degradation, liberating NFκB, and allowing its translocation to the nucleus.

Figure 5.

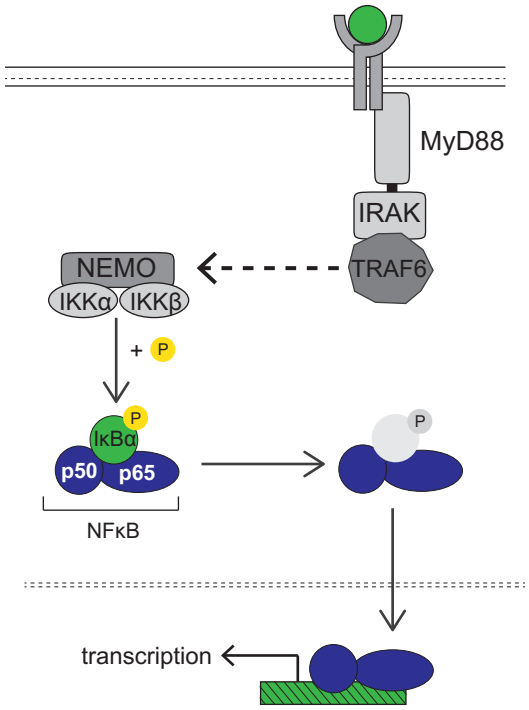

MyD88
IRAK
TRAF6
NEMO
IKKα
IKKβ
+ P
P
IκBα
p50
p65
NFκB
P
transcription

Table 2. Clinical studies examining the effects of antagonizing IL-1 signaling on glucose homeostasis. ►

Clinical studies examining the effects of antagonizing IL-1 signaling on markers of glycemia in T2D and glucose intolerant subjects. ΔHbA_{1c}: change in glycated hemoglobin. I.R.: insulin resistance. AUC OGTT: area under the curve of the glucose excursion induced during an oral glucose tolerance test. Infl.: markers of inflammation. y: years. IGT: impaired glucose tolerance. m: month. D.I.: disposition index, defined as the change in insulin secretion during the first 30 minutes of an oral glucose tolerance test as a ratio of the concomitant change in glucose concentrations. ND: not reported.

Source	Cohort	Treatment Duration	Treatment	Fasting Glucose	ΔHbA$_{1c}$	IR	AUG OGTT	Infl.
Larsen, 2007	T2D (~10y)	13 weeks, daily	anakinra or placebo	↓	-0.46%	=	↑	↓
Larsen, 2009		Larsen, 2007 + 39 week withdrawal		ND	=	=	=	=
Cavelti-Weder, 2012	T2D (~10y)	3 months, 1 dose	gevokizumab or placebo	ND	-0.85%	=	↑ 1m =3m	↓
		3 months, mutliple		ND	=	ND	ND	ND
Sloan-Lancaster, 2013	T2D (~8y)	12 weeks, weekly	LY2189102 or placebo	↓	-0.38%	=	=	↓
		above + 12 week withdrawal		=	=	ND	ND	ND
van Asseldonk, 2011	metabolic syndrome	4 weeks daily	anakinra	=	=	=	↑ D.I.	↓
Ridker, 2012	T2D (~4y)	4 months, monthly	canakinumab or placebo	=	=	=	ND	↓
Rissanen, 2012	IGT	4 weeks, 1 dose	canakinumab or placebo	=	ND	ND	↑	↓
van Poppel, 2014	IGT	4 weeks, daily	anakinra or placebo	=	=	=	↑	ND
Timper, 2015	T2D (~9y)	8 weeks, bi-weekly	MABp1	=	=	ND	ND	=
Stahel, 2015	T1D + T2D	24 weeks, 3 doses (8 weeks)	canakinumab	ND	-0.62%	ND	ND	=

1.5 SUMOYLATION

1.5.a Small ubiquitin-like modifier proteins.

Small ubiquitin-like modifier (SUMO) proteins are involved in post-translational modification of numerous cellular proteins. There are four currently identified members of the SUMO family, named SUMO1 through 4 (Geiss-Friedlander and Melchior, 2007). SUMO proteins exert their biological functions following their covalent attachment to target proteins. SUMO attachment, termed SUMOylation, requires activation of a cascade of enzymatic events (reviewed in Geiss-Friedlander and Melchior, 2007 and summarized in Figure 6). Briefly, SUMO proteins, expressed as immature pro-forms, are activated following proteolytic cleavage by SUMO proteases. Mature SUMO proteins are transferred to E1 conjugating enzymes and are then covalently attached to lysine residues of target proteins by the E2 conjugating enzyme UBC9—a process that is facilitated by E3 ligase enzymes known as protein inhibitor of activated STAT (PIAS). SUMOylation is made reversible by the sentrin/SUMO-specific proteases (SENPs), named SENP1 to 3 and SENP5 to 7 (reviewed in Yeh, 2009). Specificity to SUMO conjugates differs between SENP isoforms: whereas SENP3 and SENP5 preferentially deconjugate SUMO2/3 (Gong and Yeh, 2006), SUMO1 is preferentially deconjugated by SENP1 (Sharma et al., 2013). The consequences of SUMO conjugation are numerous, including modification of protein-DNA binding (Akar and Feinstein, 2009), protein stability (Aukrust et al., 2013; Desterro et al., 1998), and cellular localization (Li et al., 2008; Meinecke et al., 2007; Rajan et al., 2012). Furthermore, given the large number of SUMOylatable targets currently identified (Akar and Feinstein, 2009; Aukrust et al., 2013; Dai et al., 2009; Desterro et al., 1998; Feligioni et al., 2011; Kim et al., 2011; Okura et al., 1996; Pascual et al., 2005; Rajan et al., 2012), the cellular outcomes of SUMO conjugation are diverse and difficult to predict.

1.5.b SUMO1 and β-cell function.

Pancreatic islets express *SUMO1* and *SUMO3* (Dai et al., 2009). Although insulinotropic effects of SUMO1 on glucokinase (Aukrust et al., 2013) and voltage-gated K^+ channels (Dai et al., 2009) have been reported, overall, SUMO1 conjugation results in β-cell secretory dysfunction. SUMO1 impairs β-cell function downstream of granule recruitment (Dai et al., 2011) and reduces exendin-4 potentiation of glucose-stimulated insulin secretion by decreasing GLP-1 receptor signaling (Rajan et al., 2012). Conversely, the necessity of the deSUMOylating enzyme SENP1 for glucose-induced insulin secretion has been recently reported: islet-specific *Senp1*-knockout mice are characterized by reduced stimulus-induced insulin secretion and impaired glucose tolerance (Ferdaoussi et al., 2015). Consistent with the inhibitory actions of SUMO1 on secretory function, both acute infusion (Ferdaoussi et al., 2015) and overexpression (Dai et al., 2011) of SENP1 increase β-cell exocytosis under low glucose conditions.

1.5.c SUMO1 and cell survival.

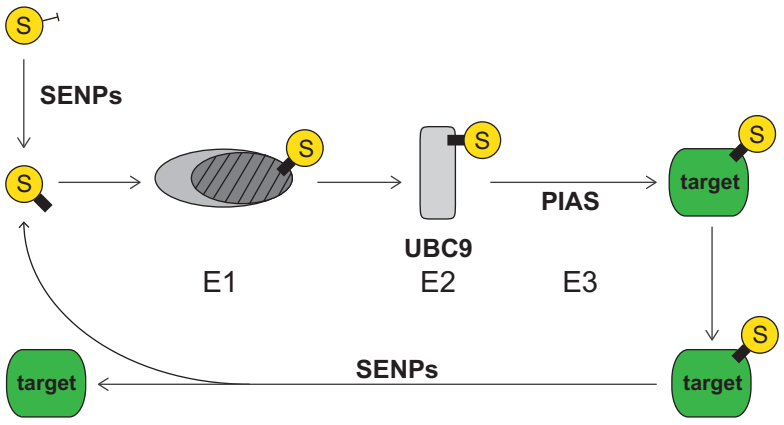
One of the earliest identified functions of SUMO1 was its ability to reduce Fas- and cytokine-induced apoptosis (Okura et al., 1996). The ability of SUMO1 conjugation to protect against various apoptotic stimuli in several cell types has since been examined. Reflective of the extensive diversity of its target protein population, SUMO1 has varied effects on cellular viability, conferring protection against heat shock- (Kim et al., 2011), cytokine- (Okura et al., 1996), and Fas ligand- (Meinecke et al., 2007; Okura et al., 1996) induced apoptosis in some cell types, while enhancing cell death in others (Feligioni et al., 2011). Although the effects of SUMOylation on pancreatic β-cell viability are unknown, that

SUMO1 inhibits activation of NFκB (Akar and Feinstein, 2009; Desterro et al., 1998; Liu et al., 2009), JNK (Kim et al., 2011), and *Nos2* transcription (Akar and Feinstein, 2009; Pascual et al., 2005) in other cell types suggests an anti-apoptotic role for SUMO1 in pancreatic β-cells (Section 1.4.b). Consistent with its cytotoxic effects in other cell types (Cimarosti et al., 2012; Li et al., 2008), these data also suggest a pro-apoptotic role for SENP1. Although SENP1 overexpression enhances β-cell secretory function (Dai et al., 2011), a reduction in β-cell viability may overshadow the stimulatory effects of SENP1. The consequences of enhanced SENP1 expression on insulin secretion and β-cell viability, however, have yet to be determined. Given the significant role of apoptosis in T2D pathogenesis (Butler et al., 2003), regulators of β-cell death, particularly those capable of protecting against inflammation-induced apoptosis, may represent alternative therapeutic targets in the preservation of β-cell mass.

Figure 6. SUMOylation. ►

SUMO is activated by SENP proteases. Following activation, SUMO attaches to E1 enzymes, is transferred to E2 enzymes, and is finally conjugated to target proteins with assistance of E3 ligase enzymes. SUMOylation is reversed by SENP proteases, which remove SUMO from target proteins.

Figure 6.



1.6 GENERAL HYPOTHESIS

The present thesis hypothesizes that *acute* IL-1 signaling is necessary for the maintenance of glucose homeostasis during brief exposures to metabolic stress. In contrast, it theorizes that *persistent* IL-1 signaling is detrimental to β-cell viability and that this occurs in a SUMOylation-regulated manner.

Collectively, this thesis hypothesizes that the consequences of IL-1 signaling on glucose homeostasis are duration-dependent, with IL-1β acting as both a beneficial and detrimental regulator of pancreatic β-cell function.

1.7 SPECIFIC AIMS

1.7.a AIM 1. Elucidate the role of IL-1 signaling in response to acute metabolic and inflammatory stress.

The necessity of IL-1 signaling in the maintenance of glucose homeostasis, under both basal conditions and in response to acute HFD or LPS-induced inflammation, was assessed in *Il1r1*-deficient mice. Mechanisms underlying the acute insulinotropic effects of IL-1β were examined in mouse and human β-cells. Metabolic factors correlating with the insulinotropic efficacy of IL-1β on human islet function were identified.

1.7.b AIM 2. Examine whether cytotoxicity induced by persistent IL-1 signaling is regulated by SUMOylation.

The role of SUMOylation as a regulator of IL-1β-induced apoptosis was evaluated using the adenovirus-mediated overexpression of SUMO1 in human islets and in an insulin secreting cell line. Conversely, the effect of deSUMOylation was determined using both an siRNA-mediated down-regulation and an adenoviral-mediated overexpression of the SUMO protease SENP1. Mechanisms underlying SUMOylation-induced cytoprotection were examined.

1.7.c AIM 3. Evaluate the cytoprotective efficacy of SUMO1 against IL-1β and isolation-induced β-cell cytotoxicity.

SUMOylation was enhanced in human β-cells and in an insulin secreting cell line using a TAT-peptide transduction strategy. The transduction efficiency and biological activity of TAT-SUMO1 was assessed using molecular and functional measurements, respectively. The ability of SUMOylation to inhibit activation of isolation-associated stress pathways was examined in an insulin secreting cell line exposed to IL-1β and in isolated human islets.

CHAPTER 2.

Interleukin-1 signaling contributes to acute islet compensation.

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Co-author contributions to the figures presented are acknowledged in the figure legends by co-author initials.

2.1 ABSTRACT

IL-1β is a well-established inducer of impaired pancreatic islet function. Despite this, findings examining IL-1R deficiency or antagonism in in vivo animal models, as well as in clinical studies of T2D patients, have produced conflicting results, indicating that the action of IL-1β on glycemic control may be pleiotropic in nature. In the present work, we find that the ability of IL-1β to amplify glucose-stimulated insulin secretion from human islets correlates with donor BMI. Islets from obese donors are sensitized to the insulinotropic effects of this cytokine, whereas the stimulatory effects of IL-1β are lost in islets from obese T2D patients, suggestive of a role for IL-1 signaling in islet compensation. Indeed, mice deficient in *Il1r1* become glucose intolerant more rapidly than their wild-type littermates and have impaired secretory responses during the acute stages of inflammatory and metabolic stress induced by the pro-inflammatory endotoxin LPS or a high-fat diet, respectively. IL-1β directly enhances β-cell insulin secretion by increasing granule docking and soluble SNARE complex formation at the plasma membrane. Together, our study highlights the importance of IL-1β signaling in islet compensation to metabolic and inflammatory stress.

2.2 INTRODUCTION

Activation of the innate immune system by metabolic stress is a prominent mediator of β-cell failure during T2D (Donath and Shoelson, 2011; Kahn et al., 2014). The detrimental effects of inflammation are mediated predominantly by the pro-inflammatory cytokine IL-1β. Inhibition of IL-1 signaling by IL-1Ra prevents β-cell cytotoxicity induced by chronic metabolic stress, restoring secretory function in pancreatic islets (Maedler et al., 2002; Sauter et al., 2008). Conversely, prolonged exposure to IL-1β, which is elevated in islets of T2D patients (Böni-Schnetzler et al., 2008; Maedler et al., 2002), promotes β-cell dysfunction (Maedler et al., 2002; Rabinovitch et al., 1990).

Despite the detrimental effects of IL-1β exposure on β-cells in vitro, circulating levels of IL-1β alone were not associated with increased T2D risk in a large, multicenter cohort (Spranger et al., 2003). Sustained inhibition of IL-1 signaling, induced by a genetic up-regulation of IL-1Ra, was also found to have no effect on diabetes risk (Interleukin 1 Genetics Consortium, 2015). Therapeutic interventions aimed at reducing IL-1 signaling in patients with T2D or metabolic syndrome have produced conflicting results (Cavelti-Weder et al., 2012; Larsen et al., 2007; Ridker et al., 2012; Rissanen et al., 2012; Sloan-Lancaster et al., 2013; van Asseldonk et al., 2011; van Poppel et al., 2014), with some studies demonstrating an inability of these therapies to significantly improve glycemic control, despite effectively reducing systemic inflammation (Ridker et al., 2012; Rissanen et al., 2012; van Asseldonk et al., 2011). Furthermore, inhibition of IL-1 signaling, induced through genetic knockout *Il1r1* (García et al., 2006) or *Il1b* (Maedler et al., 2006), induces glucose intolerance in mice. These findings suggest that the actions of IL-1β on glycemic control may be pleiotropic in nature, with IL-1 signaling exerting both positive and negative effects in vivo.

Beneficial effects of IL-1β on β-cell function and glycemic control have been reported. Acute infusion of IL-1β induces hypoglycemia (Besedovsky and del Rey, 1987; del Rey and Besedovsky, 1987; Oguri et al., 2002) and increases plasma insulin concentrations (Cornell, 1989; del Rey and Besedovsky, 1987; Ling et al., 1995). While the underlying mechanism of action is unknown, the rapid nature of the exposure indicates that the stimulatory effect of IL-1β is not due to proliferative actions. In support of this, IL-1β acts directly on β-cells to potentiate glucose-stimulated insulin secretion from perfused pancreata (Yelich, 1990; 1992), isolated islets (Arous et al., 2015; Borg and Eizirik, 1990; Comens et al., 1987; Eizirik and Sandler, 1989; Eizirik et al., 1992; 1993; 1995; Helqvist et al., 1989; Jeong et al., 2002; Palmer et al., 1989; Welsh et al., 1989; 1991; Zawalich and Diaz, 1986; Zawalich and Zawalich, 1989), and purified β-cells (Arous et al., 2015). Although several potential mechanisms of IL-1β-potentiated insulin secretion have been proposed (Arous et al., 2015; Jeong et al., 2002; McDaniel et al., 1988), the role of both intracellular Ca^{2+} and the amplification pathway in this process remain unclear.

Inflammation is induced rapidly upon exposure to metabolic stress (Lee et al., 2011), with several reports observing post-prandial elevations in inflammatory markers (de Vries et al., 2014; Esposito et al., 2002; Sage et al., 2012; van Oostrom et al., 2003). Acute hyperglycemia has been shown to increase IL-1β transcript expression in peripheral leukocytes (Sage et al., 2012). Metabolic stress also induces up-regulation of IL-1β, with an elevation of the IL-1β target gene, *Nos2*, observed as early as 3 days in white adipose tissue from high fat diet (HFD)-fed mice (Lee et al., 2011). Furthermore, both high glucose and elevated free fatty acid concentrations induce intra-islet production of IL-1β within 4 days of exposure (Böni-Schnetzler et al., 2009; Maedler et al., 2002). That mechanisms have evolved

to increase IL-1β expression early in response to metabolic stress suggests an important compensatory role of this cytokine in the acute stages of overnutrition.

This study examined the role of IL-1 signaling in the acute response to metabolic stress and inflammation, and to the mechanisms underlying IL-1β-mediated potentiation of insulin secretion. IL-1β was shown to potentiate insulin secretion from pancreatic β-cells by increasing exocytosis subsequent to enhanced insulin granule docking at the plasma membrane. While the stimulatory efficacy of IL-1β was found to positively correlate with BMI in non-diabetic human islets, IL-1β was unable to potentiate glucose-stimulated insulin secretion in islets from obese T2D donors. Genetic knockout of *Il1r1* exacerbated glucose intolerance in mice following a short-term exposure to HFD and reduced glucose sensitivity induced by acute LPS injection. The impairment in glucose tolerance observed in both HFD- and LPS-treated mice was associated with the reduced ability of glucose to stimulate insulin secretion in vivo, demonstrating a beneficial role of IL-1 signaling in acute islet compensation to metabolic stress.

2.3 MATERIALS AND METHODS

2.3.a Cells and cell culture.

Islets from male C57BL/6 or transgenic mice were isolated by collagenase digestion and cultured in RPMI 1640 containing 11.1 mmol/l glucose with 10% FBS and 100 U/ml of penicillin/streptomycin. Human islets were isolated from donor pancreata at the Alberta Diabetes Institute IsletCore (http://www.bcell.org/IsletCore.html) at the University of Alberta (Edmonton, Alberta, Canada) or the Clinical Islet Laboratory at the University of Alberta and were cultured in low-glucose (5.5 mmol/l) DMEM with L-glutamine, 100 mg/ml sodium pyruvate, 10% FBS, and 100 units/ml penicillin/streptomycin. In total, islets from 22 human donors were examined in this study (age: 52.6 ± 2.8 years; BMI: 29 ± 1.4 kg/m^2; HbA_{1c}: 5.8 ± 0.16), of which 3 were considered diabetic by patient clinical history (Table 3). For single cell experiments, human or mouse islets were dispersed by shaking in cell dissociation buffer (Gibco, Thermo Scientific) and plated in 35-mm culture dishes. INS 832/13 cells, a rat insulinoma cell line from C. Newgard (Duke University, Durham, North Carolina, USA), were cultured in RPMI 1640 containing 11.1 mmol/l glucose with 10% FBS, 10 mmol/l HEPES, 0.29 mg/ml L-glutamine, 1 mmol/l sodium pyruvate, 50 μmol/l β-mercaptoethanol, and 100 U/ml of penicillin/streptomycin. Islets, INS 832/13 cells, or dispersed cells were cultured at 37°C and 5% CO_2.

2.3.b Insulin secretion assay.

Measurements were performed at 37°C in KRB solution containing (in mmol/l): 115 NaCl, 5 KCl, 24 $NaHCO_3$, 2.5 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, and 0.1% BSA (pH 7.4). For static measurements, intact mouse or human islets were pre-incubated for 2 hours in either

2.8 or 1.0 mmol/l glucose-KRB, respectively. Islets were transferred to fresh KRB solution containing 2.8 or 1.0 mmol/l glucose for 1 hour, followed by incubation for 1 hour in 16.7 mmol/l glucose-KRB. Supernatant fractions were collected, and islets were lysed in buffer containing 1.5% concentrated hydrochloric acid, 23.5% acetic acid, and 75% ethanol for assay of insulin content. Samples were stored at -20°C and assayed for insulin via insulin detection kits (Meso Scale Discovery). Unless otherwise indicated, intact mouse and human islets were treated with human recombinant IL-1β (10 ng/ml; Sigma-Aldrich) for 4 or 1 hour, respectively, due to differences in responses observed between species (Figure 7, A and B). Latrunculin B (10 μmol/l; Sigma-Aldrich) was added to high glucose stimulation (Figure 11, C and D); in human islet experiments, IL-1β was added 1 hour prior and in addition to latrunculin B treatment (Figure 11D). For perifusion experiments, intact mouse islets were perfused (0.1 ml/min.) with 2.8 mmol/l glucose for 30 minutes (as a pre-incubation step) and then with glucose (2.8 or 16.7 mmol/l) and either vehicle or recombinant human IL-1β (10 ng/ml), as indicated.

2.3.c $[Ca^{2+}]_i$ measurements.

Prior to recordings, intact mouse islets were pre-treated for 4 hours with vehicle (0h or 0h + IL-1β) or human recombinant IL-1β (10 ng/ml; 4h + IL-1β). Islets were incubated for 45 minutes with 3 μmol/l Fura-2, AM (Invitrogen) and 0.06% pluronic acid (Invitrogen) in solution containing (in mmol/l): 130 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 5 $NaHCO_3$, and 10 HEPES (pH 7.4) in the presence of vehicle (0h or 0h + IL-1β) or IL-1β (4h + IL-1β). Islets were imaged in 0.5 mmol/l glucose at 37°C with constant bath perfusion. Glucose was increased to 11 mmol/l and IL-1β was added (0h + IL-1β) or maintained (4h + IL-1β), as

indicated. Imaging was performed with a Stallion Imaging System (Olympus) and acquired with Ratio Cam software (Metamorph; Molecular Devices). Excitation was at 340 and 380 nm, and emission was collected using 510 nm bandpass filter (Semrock). The glucose-stimulated $[Ca^{2+}]_i$ response (GSCa) was calculated as the fold increase in the Fura-2 ratio following 11 mmol/l glucose versus the ratio at 0.5 mmol/l glucose.

2.3.d Electrophysiology.

Dispersed mouse islets were plated in 35-mm culture dishes. Prior to electrophysiological recordings, cells were incubated in RPMI 1640 media with 2.8 mmol/l glucose for 1 hour. Solutions used for capacitance measurements are previously described (Dai et al., 2011). Vehicle or human recombinant IL-1β (10 ng/ml) were added to the extracellular bath solution for the duration of the recordings. Cells were patched following a 15- to 60-minute treatment with vehicle or IL-1β. The standard whole-cell technique with the sine+DC lockin function of an EPC10 amplifier and Patchmaster software (HEKA Electronik, Lambrecht/Pfalz) was used. Experiments were performed at 32-35°C. The Ca^{2+} charge entry was calculated as the area under the curve of the Ca^{2+} current upon a single 500 ms depolarization from -70 to 0 mV normalized to cell size.

2.3.e Actin staining.

Dispersed human or mouse islet cells were plated onto untreated coverslips. Cells were pre-incubated with 1.0 or 2.8 mmol/l glucose-KRB for 2 hours prior to treatment, respectively. Dispersed cells were treated with vehicle or human recombinant IL-1β (10 ng/ml), with latrunculin B (10 μmol/l, 2 minutes; Sigma-Aldrich), or with high glucose (16.7 mmol/l, 15 minutes), as indicated. To prevent any adverse effects of prolonged incubation at

low glucose, 4-hour treatments of mouse cells were initiated 2 hours prior to, and maintained throughout, low-glucose-KRB pre-incubation. Immediately following treatments, cells were fixed in 10% Shandon Zinc Formal-Fixx (Thermo Scientific). Cells were stained for insulin with rabbit anti-insulin primary antibody (sc-9168, 1:200; Santa Cruz Biotechnology Inc.), followed by an anti-rabbit Alexa Fluor 594 (A11037, 1:200; Molecular Probes) and stained for filamentous actin (F-actin) with Alexa Fluor 488-conjugated phalloidin (A12379; Molecular Probes). Coverslips were mounted using ProLong Gold Antifade Reagent (Molecular Probes). Visualization was on a Perkin Elmer UltraView ERS spinning disk confocal (PerkinElmer) using a 40x/1.3 oil immersion objective. Fluorophores were stimulated using 488 nm (phalloidin) and 561 nm (insulin), and images were recorded on a Hamamatsu 9100 EMCCD with lateral resolution of 0.2 μm/pixel. Images were acquired using Volocity software (PerkinElmer) and analyzed using ImageJ software (http://imagej.nih.gov/ij/). Four cross-section intensity measurements of F-actin were obtained and averaged for each cell. Only insulin-positive cells were analyzed.

2.3.f Electron microscopy.

Intact mouse islets were pre-incubated in 2.8 mmol/l KRB for 2 hours prior to treatment with human recombinant IL-1β (10 ng/ml) or high glucose (16.7 mmol/l), as indicated. Immediately following treatments, cells were fixed in a 2X fixative buffer containing 4% glutaraldehyde, 0.2 mol/l sucrose, and 4 mmol/l $CaCl_2$ in 0.16 mol/l sodium cacodylate (pH 7.4) and processed as previously described (Cho et al., 2007). Images were acquired using a Hitachi H-7650 transmission electron microscope equipped with a 16 megapixel EMCCD XR111 camera (Advanced Microscopy Techniques) and AMT version

600 imaging software (Advanced Microscopy Techniques). Images were analyzed using ImageJ software (http://imagej.hin.gov/ij/).

2.3.g Immunoblotting and immunoprecipitation.

INS 832/13 cells were pre-incubated for 2 hours in 0 mmol/l glucose-KRB and subsequently treated for 30 minutes with human recombinant IL-1β (10 ng/ml). Following treatment, cells were lysed in buffer containing (in mmol/l): 20 Tris-HCl (pH 7.5), 150 NaCl, 1 EGTA, 1 EDTA, 1% Triton-X100, and protease inhibitor cocktail (Set V; Millipore). For immunoprecipitation, 500-700 µg of total cell lysates were incubated with 2 µg of normal mouse IgG (sc-2025; Santa Cruz Biotchenology Inc.) or mouse anti-syntaxin-1 (sc73098; Santa Cruz Biotechnology Inc.) and 30 µl of 50% protein G slurry (GE Life Sciences) overnight at 4°C. Immunoprecipitates were washed 5 times with lysis buffer. These or whole cell lysates were separated using SDS-PAGE, transferred to polyvinylidene difluoride membranes (Millipore), probed with primary antibodies (anti-SNAP25 [5308S, 1:1000; Cell Signaling Technology]; anti-syntaxin-1 [sc-73098, 1:1000; Santa Cruz Biotechnology Inc.]) and detected with appropriate peroxidase-conjugated secondary antibodies (Amersham). Images were acquired using a ChemiDoc MP System (Bio-Rad) and analyzed using Image Lab Software 5.2.1 (Bio-Rad).

2.3.h Transgenic animals and metabolic studies.

Il1r1-deficient mice (B6.129S7-Il1r1^{tm1Imx}/J) and their C57BL/6J controls were purchased from The Jackson Laboratory. Experiments were conducted on male littermates between 10-14 weeks of age, unless otherwise indicated. Genotypes were determined using primers (NeoR cassette forward 5'-CTGAATGAACTGCAGGACGAGGCAG-3'; reverse 5'-

GGCCACAGTCGATGAATCCAGAAAAGC-3'. IL-1 receptor forward 5'- ATTTCA GCATGTGTTTCCACACTCCCTCA-3'; reverse 5'-TACGCCCTCCCACTTGAACAA CTTATTTGG-3') and the REDE-N-Amp tissue PCR kit (Sigma-Aldrich) on ear notches. Where indicated, mice were fed a HFD (60% kcal from fat, F3283; Bio-Serve) for 2, 9, or 30 days or were given an intraperitoneal injection of either 2 mg/kg LPS (Sigma-Aldrich) or PBS vehicle control 6 hours prior to metabolic studies. Glucose tolerance tests were performed on animals fasted for 3 or 6 hours, as indicated in figure legends, by intraperitoneal injection of dextrose (1 g/kg). Blood was collected at times indicated, assayed for glucose using a glucose meter (OneTouch), and stored in Microvette 100 Li Heparin (Sarstedt) tubes. Samples were centrifuged at 4°C for 10 minutes at 9,330 *g*, and supernatant was collected and assayed for insulin using insulin detection kits (Meso Scale Discovery). Insulin tolerance tests were performed on mice fasted for 3 or 6 hours, as indicated in figure legends, by intraperitoneal injection of 1 U/kg Humulin R (Lilly).

2.3.i Statistics.

Data were analyzed using FitMaster (HEKA Electronik) or GraphPad Prism v6.0c and were compared by unpaired Student's *t* test or by 1-, 2-way, or repeated-measures ANOVA followed by a post hoc *t* test using the Tukey or Sidak method, as indicated in figure legends. Outliers were identified and removed using Grubbs' test for outliers except for one animal that was removed from data set in Figure 14A, as its body weight differed by greater than 10% of the group mean. Associations between variables were analyzed using Pearson correlation co-efficients, and lines of best fit were obtained through linear regression. Data are expressed as means ± SEM, where $P<0.05$ is considered significant.

2.4 RESULTS

2.4.a The stimulatory capacity of IL-1β is positively correlated with BMI of non-diabetic donors but is absent in T2D islets.

Consistent with previous reports (Arous et al., 2015; Borg and Eizirik, 1990; Comens et al., 1987; Eizirik and Sandler, 1989; Eizirik et al., 1992; 1993; 1995; Helqvist et al., 1989; Jeong et al., 2002; Palmer et al., 1989; Welsh et al., 1989; 1991; Zawalich and Diaz, 1986; Zawalich and Zawalich, 1989), acute exposure to IL-1β (10 ng/ml) enhanced glucose-stimulated insulin secretion from mouse (Figure 7A) and human (Figure 7B) islets. IL-1β had no effect on islet insulin content and did not affect secretion at low glucose (data not shown).

Substantial variability in the ability of IL-1β to potentiate glucose-stimulated insulin secretion between individual human islet preparations was observed (Table 3), prompting the examination of associations between the insulinotropic capacity of IL-1β and several anthropometric and metabolic variables. The potentiation index of IL-1β, defined as the ratio of insulin secretion induced by high glucose in the presence of IL-1β to insulin secretion induced by high glucose alone, positively correlated to BMI in non-diabetic islets (r=0.63; Figure 7C). The ability of IL-1β to potentiate glucose-stimulated insulin secretion was not associated with the stimulation index of glucose (r=-0.42; Figure 7D), glycated hemoglobin (Hb_{A1c}; r=-0.17; Figure 7E), or age (r=0.25; data not shown). The stimulatory capacity of IL-1β was also assessed in islets from T2D donors. A significant difference in the potentiation index of IL-1β between obese and non-diabetic obese T2D donors was observed (Figure 7F). While mean BMI was higher in the T2D donors than the non-diabetic donors (Table 4), BMI was no longer correlated to the potentiation index of IL-1β when diabetic donors were included in the analysis (P=0.73; r=0.09; data not shown). Contrary to the significant

insulinotropic effect of IL-1β observed in non-diabetic islets (Figure 7B, G), IL-1β did not potentiate glucose-stimulated insulin secretion from obese, T2D donors (Figure 7H).

IL-1 signaling was not required for optimal secretory function under normal conditions (Figure 8), as no significant differences in β-cell Ca^{2+} currents, intracellular Ca^{2+} handling, granule trafficking, exocytosis, or glucose-stimulated insulin secretion were observed in mice deficient in *Il1r1* (Figure 9).

Figure 7. The stimulatory capacity of IL-1β is correlated with BMI and is absent in T2D islets. ►

A, B: Insulin secretion normalized to percent insulin content from **(A)** mouse (*n*=15, 17, 17, =17; 6 experiments); data collection by AFS or **(B)** human islets (*n*=18, 18, 18, 18; 6 donors) following exposure to IL-1β, as indicated, and stimulated with 1.0, 2.8, or 16.7 mmol/l glucose. **C-E:** The potentiation index of 1-hour IL-1β treatment plotted against **(C)** BMI (kg/m^2) (*n*=14 donors), **(D)** glucose stimulation index (*n*=14 donors), and **(E)** glycated hemoglobin (HbA_{1c}) (*n*=13 donors) in non-diabetic human islet donors. **F:** Comparison of IL-1β potentiation index between T2D and control islets according to BMI tertiles [lean < 25; overweight 25 – 30; and obese > 35 kg/m^2] (*n*=3, 7, 4, 3 donors). **G, H:** Insulin secretion normalized to percent insulin content from **(G)** non-diabetic (*n*=41, 36, 39, 39; 14 donors); data collection by CH or AB, or **(H)** T2D human donors (*n*=9, 8, 9, 8; 3 donors) treated for 1 hour with vehicle or IL-1β (10 ng/ml) and stimulated with either 1.0 or 16.7 mmol/l glucose. *n* values correspond to data points from left to right, respectively. Data are mean ± SEM and were compared with **(A, B, G, H)** 2-way ANOVA followed by Tukey post-test, **(C-E)** Pearson correlation coefficients, or **(F)** 1-way ANOVA followed by Tukey post-test. *P<0.05, **P<0.01, ***P<0.001, as indicated. G: glucose.

Figure 7.

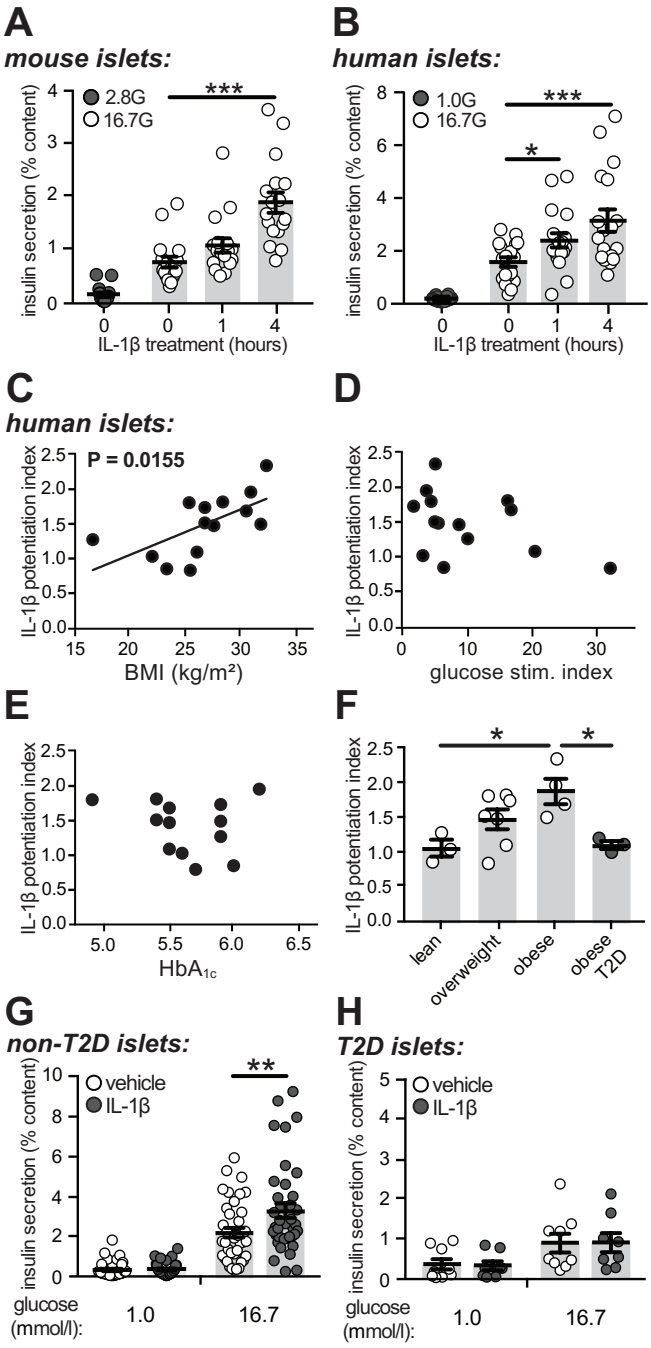

A
mouse islets:
2.8G
16.7G
***
insulin secretion (% content)
IL-1β treatment (hours)
B
human islets:
1.0G
16.7G
***
*
insulin secretion (% content)
IL-1β treatment (hours)
C
human islets:
P = 0.0155
IL-1β potentiation index
BMI (kg/m²)
D
IL-1β potentiation index
glucose stim. index
E
IL-1β potentiation index
HbA1c
F
*
*
IL-1β potentiation index
lean
overweight
obese
obese T2D
G
non-T2D islets:
vehicle
IL-1β
**
insulin secretion (% content)
glucose (mmol/l):
1.0
16.7
H
T2D islets:
vehicle
IL-1β
insulin secretion (% content)
glucose (mmol/l):
1.0
16.7

Figure 8. *Il1r1*-deficiency has a minor effect on glucose-stimulated insulin secretion. ►

A: Insulin secretion normalized to fold increase over baseline at low glucose from wild-type (+/+) or *Il1r1*-knockout (-/-) islets exposed to 2.8 or 16.7 mmol/l glucose and treated with IL-1β (10 ng/ml) as indicated (left) (*n*=7, 8 mice). Change in area under the curve (ΔAUC) of the response to high glucose (middle) (*n*=7, 7 mice) and to high glucose and IL-1β (right) (*n*=7, 8 mice) are shown. Data collection by AFS. **B:** Average insulin secretion during 2.8 mmol/l glucose baseline from wild-type (+/+) or *Il1r1*-knockout (-/-) islets (*n*=7, 8 mice). *n* values correspond to data points from left to right, respectively. Data are mean ± SEM and were compared with **(A)** a two-tailed Student's *t*-test. *P<0.05 as indicated.

Figure 8.

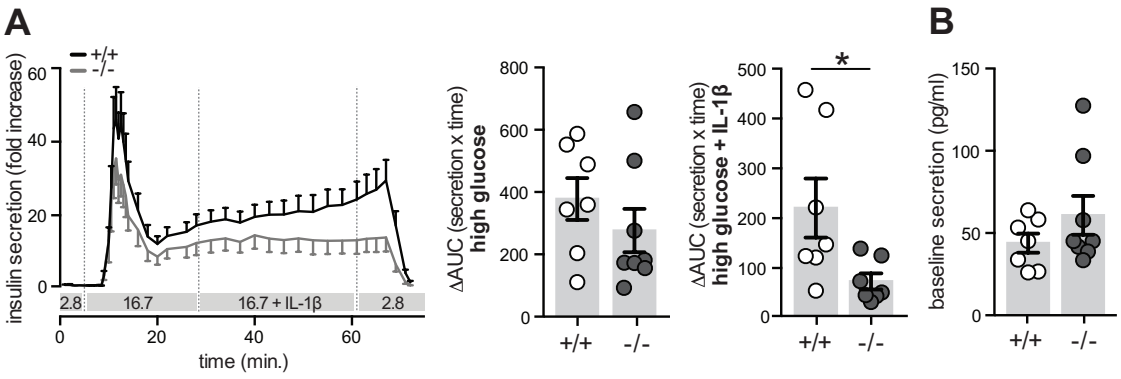

A
+/+
-/-
insulin secretion (fold increase)
60
40
20
0
2.8
16.7
16.7 + IL-1β
2.8
0
20
40
60
time (min.)
ΔAUC (secretion x time)
high glucose
800
600
400
200
0
+/+
-/-
ΔAUC (secretion x time)
high glucose + IL-1β
500
400
300
200
100
0
*
+/+
-/-
B
baseline secretion (pg/ml)
150
100
50
0
+/+
-/-

Figure 9. *Il1r1*-deficiency impairs F-actin dynamics. ►

A: Representative PCR-based genotyping using genomic DNA (from ear notches) or mice with indicated genotypes. **B:** Quantification of exocytotic responses measured as increases in cell membrane capacitance following a single depolarization from -70 to 0 mV in dispersed mouse β-cells from wild-type (+/+) or *Il1r1*-knockout (-/-) mice in the presence of either 2.8 or 16.7 mmol/l glucose (*n*=16, 15, 23, 20; 3 experiments); data collection by XD. **C:** Responses in intracellular Ca^{2+} ($[Ca^{2+}]_i$) from wild-type (+/+) or *Il1r1*-knockout (-/-) islets following glucose-stimulation (arrow; left) and change in area under the curve (ΔAUC) of $[Ca^{2+}]_i$ responses (right) (*n*=9, 9; 3 experiments). **D:** Quantification of average peak F-actin intensities of dispersed β-cells from wild-type (+/+) or *Il1r1*-knockout (-/-) mice treated with 2.8 or 16.7 mmol/l glucose (10 minutes) or latrunculin B (lat. B; 10 μmol/l; 2 minutes) (*n*=45, 45, 46, 53, 45, 47; 3 experiments); data collection by AFS. **E:** Representative electron micrographs of mouse β-cells from wild-type (+/+) or *Il1r1*-knockout mice (left), and quantification of the number of granules per micron of plasma membrane (right) (*n*=40, 35, 40, 35, 41, 35; 3 experiments). Experiments were performed in 2.8 mmol/l glucose. Black dashed line indicates plasma membrane. Scale bar represents 500 nm. Data analysis by LS under supervision of CH. **F:** Exocytotic responses from single wild-type (+/+) or *Il1r1*-knockout (-/-) mouse β-cells measured as an increase in cell membrane capacitance during a train of membrane depolarizations from -70 to 0 mV in 16.7 mmol/l glucose (*n*=32, 35; 3 experiments); data collection by XD. *n* values correspond to data points from left to right, respectively. Data are mean ± SEM and were compared with **(D)** 2-way ANOVA with Tukey post-test. $^{\#}P<0.05$, $^{\#\#}P<0.01$ versus 2.8 mmol/l wild-type control; $^{*}P<0.05$, $^{***}P<0.005$ as indicated.

Figure 9.

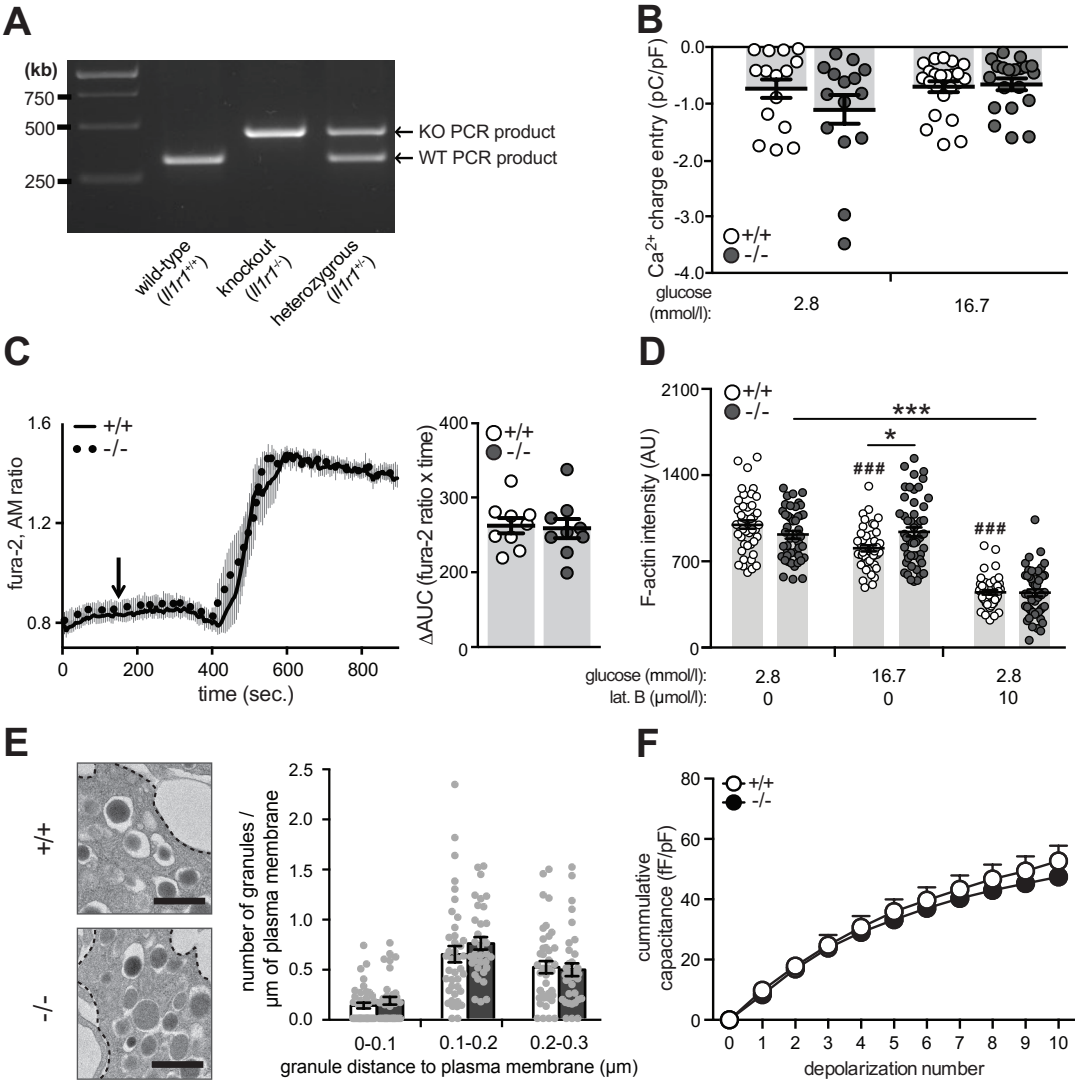

A
(kb)
750
500
250
KO PCR product
WT PCR product
wild-type (Ilf1r+/+)
knockout (Ilf1r-/-)
heterozygous (Ilf1r+/-)
B
Ca2+ charge entry (pC/pF)
0.0
-1.0
-2.0
-3.0
-4.0
+/+
-/-
glucose (mmol/l):
2.8
16.7
C
fura-2, AM ratio
1.6
1.2
0.8
+/+
-/-
0
200
400
600
800
time (sec.)
ΔAUC (fura-2 ratio x time)
400
300
200
0
D
F-actin intensity (AU)
2100
1400
700
0
***
*
###
###
glucose (mmol/l):
lat. B (μmol/l):
2.8
0
16.7
0
2.8
10
E
+/+
-/-
number of granules / μm of plasma membrane
2.5
2.0
1.5
1.0
0.5
0.0
0-0.1
0.1-0.2
0.2-0.3
granule distance to plasma membrane (μm)
F
cummulative capacitance (fF/pF)
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10
depolarization number

Table 3. Human islet donor information.

Donor	Age (years)	Sex	BMI (kg/m^2)	HbA_{1c} (%)	IL-1β Index	Type 2 Diabetic
H1744	56	n/a	16.7	5.9	1.27	-
H1732	61	male	27.5	5.5	1.47	-
H1919	n/a	n/a	26.7	5.9	1.73	-
R066	44	male	32.2	n/a	2.33	-
R067	60	male	26.0	5.5	1.09	-
R072	54	female	30.8	6.2	1.95	-
R073	74	female	28.3	5.4	1.81	-
R140	49	female	22.0	5.6	1.03	-
R141	56	female	33.2	5.5	0.99	yes
R142	63	female	25.3	4.9	1.80	-
R150	42	male	31.7	5.9	1.49	-
R151	46	female	26.7	5.4	1.51	-
R152	54	female	42.6	8.3	1.19	yes
R154	57	female	40.9	7.2	1.10	yes
R157	60	female	23.3	6.0	0.85	-
R159	60	male	30.4	5.5	1.68	-
R160	27	male	25.4	5.7	0.83	-

Individual characteristics of human donors assessed in Figure 7C-E. HbA_{1c}: glycated hemoglobin. IL-1β Index: IL-1β potentiation index, defined as the ratio of insulin secretion induced by 16.7 mmol/l glucose in the presence of IL-1β to the insulin secretion induced by 16.7 mmol/l glucose alone. n/a: not available.

Table 4. Summary characteristics of human islet donors.

	Age (years)	BMI (kg/m^2)	HbA_{1c} (%)	IL-1β Index
lean [<25 kg/m^2]:				
average	55.0	20.7	5.8	1.05
SEM	± 3.21	± 2.02	± 0.12	± 0.122
overweight [25 – 30 kg/m^2]:				
average	55.2	26.6	5.5	1.46
SEM	± 6.71	± 0.414	± 0.12	± 0.142
obese [>30 kg/m^2]:				
average	50.0	31.3	5.9	1.86
SEM	± 4.24	±0.411	± 0.20	± 0.182
obese, T2D [>30 kg/m^2]:				
average	55.7	38.9	7.0	1.09
SEM	± 0.882	± 2.89	± 0.81	± 0.058

Mean characteristics of human donors studied in Figure 7C-E. HbA_{1c}: glycated hemoglobin. T2D: type 2 diabetic. IL-1β Index: IL-1β potentiation index, defined as the ration of insulin secretion induced by 16.7 mmol/l glucose in the presence of IL-1β to the insulin secretion induced by 16.7 mmol/l glucose alone. SEM: standard error of the mean. BMI: body mass index.

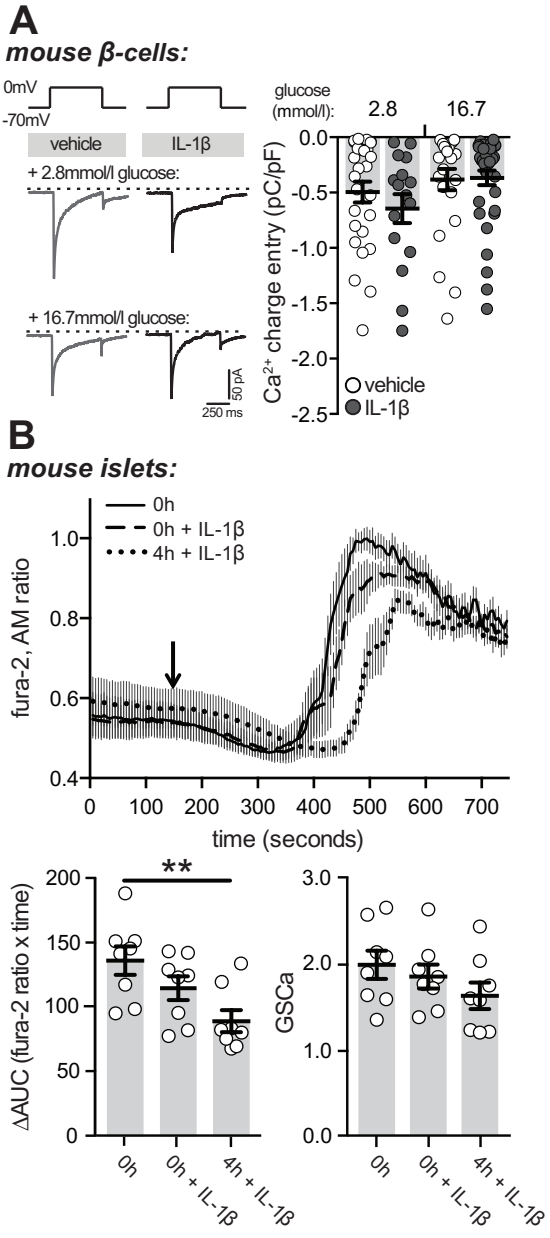
2.4.b The stimulatory effect of IL-1β is independent of changes $[Ca^{2+}]_i$.

One of several proposed mechanisms underlying the IL-1β-mediated potentiation of insulin secretion is an IL-1β-induced rise in intracellular Ca^{2+} concentrations $[Ca^{2+}]_i$ (Arous et al., 2015; Borg and Eizirik, 1990; McDaniel et al., 1988). While it is known that the insulinotropic effect of IL-1β requires Ca^{2+} flux into the β-cell (Eizirik and Sandler, 1989; Eizirik et al., 1995), whether the stimulatory effects of IL-1β are due to an increase in $[Ca^{2+}]_i$ is debated (Arous et al., 2015; Borg and Eizirik, 1990; Helqvist et al., 1989; Welsh et al., 1989). To provide further insight into the mechanism of IL-1β-stimulated insulin release, Ca^{2+} currents and $[Ca^{2+}]_i$ were measured following treatment with IL-1β. Acute treatment of isolated mouse β-cells with IL-1β (10 ng/ml; described in methods) did not affect Ca^{2+} currents under low or high glucose conditions (Figure 10A) and did not increase $[Ca^{2+}]_i$ in intact mouse islets (Figure 10B). Rather, a 4-hour pre-treatment of mouse islets with IL-1β (10 ng/ml; 4h + IL-1β) caused a slight, yet significant, impairment in glucose-stimulated $[Ca^{2+}]_i$ handling (Figure 10B), likely an early manifestation of the impairment in $[Ca^{2+}]_i$ that is induced by prolonged exposure to IL-1β (Figure 4; Dula et al., 2010). Together, these findings demonstrate that IL-1β can potentiate insulin secretion at sites downstream of $[Ca^{2+}]_i$ entry.

Figure 10. The stimulatory effect of IL-1β is independent in changes of $[Ca^{2+}]_i$. ►

A: Representative (left) and quantified (right) traces of Ca^{2+} currents obtained by a single depolarization from -70 to 0 mV in dispersed mouse β-cells treated with vehicle or IL-1β (10 ng/ml) in the presence of either 2.8 or 16.7 mmol/l glucose (*n*=27, 16, 24, 36 cells; 3 experiments); data collection by XD. **B:** Responses in intracellular Ca^{2+} ($[Ca^{2+}]_i$) from mouse islets following pre-incubation with IL-1β (10 ng/ml) for 4 hours (4h + IL-1β) or with acute IL-1β treatment (0h + IL-1β) in conjunction with glucose stimulation (11 mmol/l, arrow; top). Change in area under the curve (ΔAUC) of $[Ca^{2+}]_i$ responses (bottom left) (*n*=8, 8, 8 islets; 3 experiments) and fold increase in glucose-stimulated $[Ca^{2+}]_i$ (GSCa) responses (bottom right) (*n*=8, 8, 8 islets; 3 experiments). *n* values correspond to data points from left to right, respectively. Data are mean ± SEM and were compared with **(A)** 2-way or **(B)** 1-way ANOVA followed by **(A, B)** Tukey post-test. *P<0.05, **P<0.01, as indicated.

Figure 10.


A
mouse β-cells:
0mV
-70mV
vehicle
IL-1β
+ 2.8mmol/l glucose:
+ 16.7mmol/l glucose:
50 pA
250 ms
glucose (mmol/l):
2.8
16.7
Ca2+ charge entry (pC/pF)
vehicle
IL-1β
B
mouse islets:
0h
0h + IL-1β
4h + IL-1β
fura-2, AM ratio
time (seconds)
ΔAUC (fura-2 ratio x time)
**
GSCa
0h
0h + IL-1β
4h + IL-1β

2.4.c Acute treatment with IL-1β-induced F-actin depolymerization.

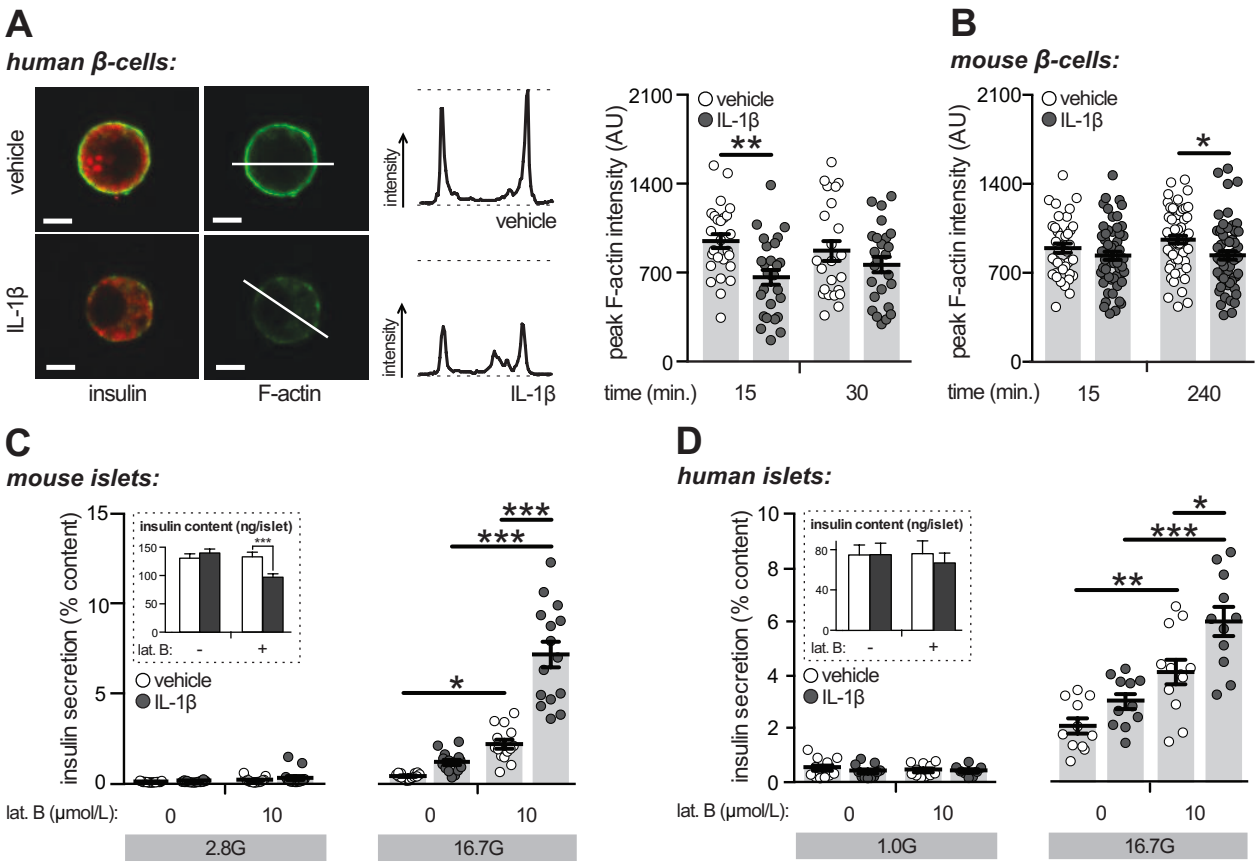
In β-cells, F-actin forms a cortical web below the plasma membrane, functioning as a barrier between granules and the plasma membrane. Disruption of the cortical F-actin barrier enhances insulin secretion secondary to increased insulin granule access to the plasma membrane (Kalwat and Thurmond, 2013; Thurmond et al., 2003). Findings that acute exposure to IL-1β alters cytoskeletal organization in rat β-cells (Arous et al., 2015) suggests that IL-1β-induced effects on actin remodeling may underlie its stimulatory effects. Treatment with IL-1β (10 ng/ml) induced F-actin depolymerization in human (Figure 11A) and mouse (Figure 11B) β-cells. This process was independent of glucose stimulation, as experiments were performed in non-stimulatory glucose concentrations (2.8 mmol/l). Indeed, while IL-1β enhances insulin secretion only in the presence of stimulatory glucose, cytoskeletal remodeling induced by IL-1β has been observed in both low- and high-glucose conditions in rat β-cells (Arous et al., 2015).

To determine if the potentiation of glucose-stimulated insulin secretion by IL-1β extends beyond its ability to depolymerize F-actin, mouse and human islets were pre-incubated with IL-1β (10 ng/ml; 4 and 1 hours, respectively) and treated with the potent F-actin depolymerizing agent, latrunculin B (10 μmol/l). IL-1β enhanced latrunculin B-potentiated glucose-stimulated insulin secretion from mouse (Figure 11C) and human (Figure 11D) islets, indicating that IL-1β is capable of enhancing glucose-stimulated insulin secretion through an F-actin-independent mechanism. While a modest, yet significant, reduction in insulin content was observed in mouse islets treated with IL-1β and latrunculin B (Figure 11C), similar effects on insulin release were observed when secretion was normalized per islet rather than to insulin content (data not shown).

Figure 11. Acute IL-1β treatment induces F-actin depolymerization. ►

A: Dispersed human β-cells treated with vehicle or IL-1β (10 ng/ml) in the presence of 2.8 mmol/l glucose, as indicated, and stained for insulin (red) or F-actin (green). Representative images (left; 15-minute treatment), example line scan analysis (middle), and quantification of average peak F-actin intensities (right) as arbitrary units (AU) are shown (*n*=26, 27, 23, 25; 4 donors). Scale bars represent 5 microns. **B**: As in **(A)**, but with dispersed mouse β-cells (*n*=39, 58, 58, 60 cells; 3 experiments). Data collection by LS under supervision of CH. **C:** Insulin secretion normalized to percent insulin content from mouse islets treated with IL-1β (10 ng/ml; 4 hours) and/or latrunculin B (lat. B; 10 μmol/l; 1 hour) and stimulated with 2.8 or 16.7 mmol/l glucose (*n*=15, 14, 15, 15, 14, 15, 14, 15; 5 experiments). Insulin contents are shown (inset); data collection by AFS. **D:** As in **(C)**, but with human islets treated with IL-1β (2 hours) and lat. B (1 hour) (*n*=12, 11, 11, 8, 11, 11, 12, 11; 4 donors) and stimulated with 1.0 or 16.7 mmol/l glucose. *n* values correspond to data points from left to right, respectively. Data are mean ± SEM and were compared with 2-way ANOVA followed by Tukey post-test. *P<0.05, **P<0.01, ***P<0.001, as indicated. G: glucose.

Figure 11.


A
human β-cells:
vehicle
IL-1β
insulin
F-actin
intensity
vehicle
intensity
IL-1β
peak F-actin intensity (AU)
2100
1400
700
0
vehicle
IL-1β
**
time (min.)
15
30
B
mouse β-cells:
peak F-actin intensity (AU)
2100
1400
700
0
vehicle
IL-1β
*
time (min.)
15
240
C
mouse islets:
insulin secretion (% content)
15
10
5
0
insulin content (ng/islet)
150
100
50
0
***
lat. B:
-
+
vehicle
IL-1β
lat. B (μmol/L):
0
10
2.8G
***
***
*
0
10
16.7G
D
human islets:
insulin secretion (% content)
10
8
6
4
2
0
insulin content (ng/islet)
80
40
0
lat. B:
-
+
vehicle
IL-1β
lat. B (μmol/L):
0
10
1.0G
*
***
**
0
10
16.7G

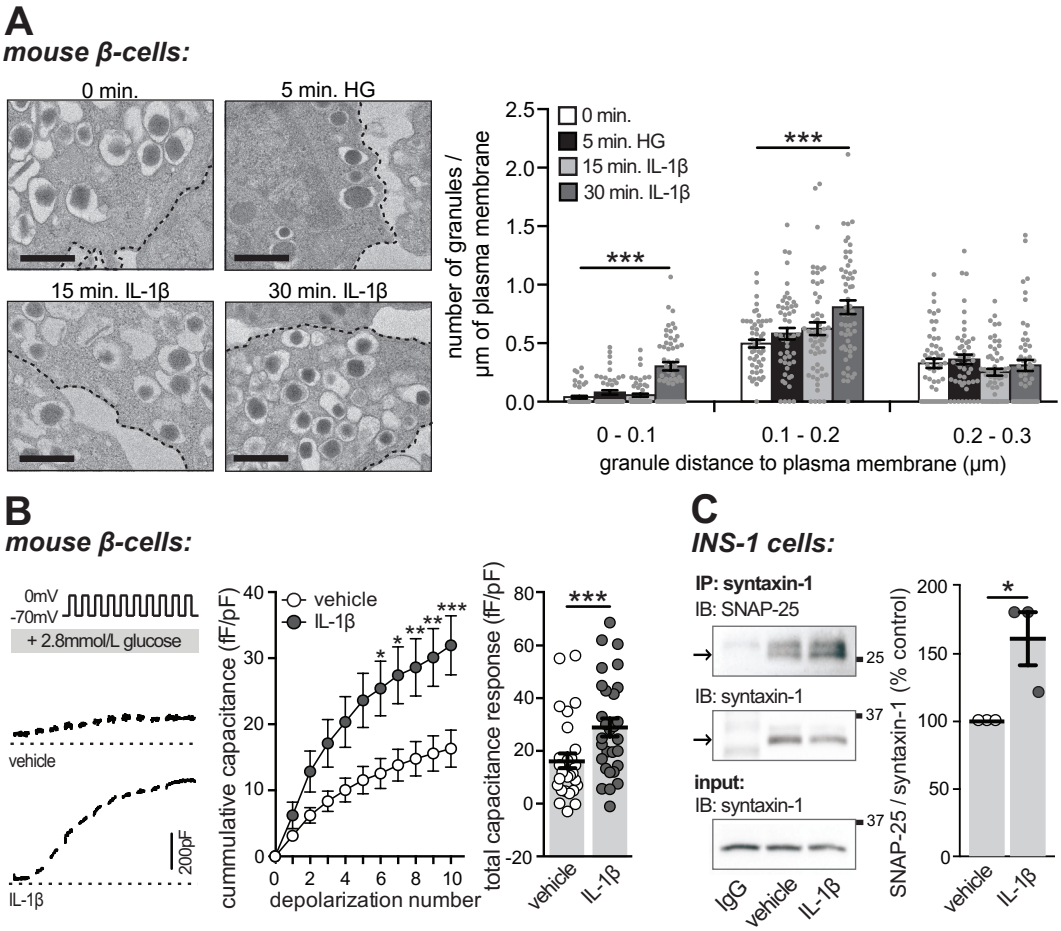
2.4.d Acute IL-1β treatment increases the number of docked insulin granules.

A lack of effect of IL-1β on $[Ca^{2+}]_i$ response suggests a role for IL-1β in regulating downstream events in insulin secretion. Ca^{2+} triggers insulin release from pancreatic β-cells through a regulated secretory pathway that depends on the recruitment, docking, and fusion of insulin granules to the plasma membrane. This process is facilitated by the formation SNARE protein complexes, which are known mediators of regulated β-cell exocytosis (Gaisano, 2014). Distance measurements between dense core granules and plasma membranes in electron micrographs of β-cells were used to assess insulin granule trafficking. Granules whose centers are located within 200 nm of the plasma membrane were considered docked (Cai et al., 2012; Gomi et al., 2005; Kasai et al., 2005). A 30-minute exposure to IL-1β (10 ng/ml) increased the number of granules less than 100 nm and within 100-200 nm of the plasma membrane in mouse β-cells, where the numbers of granules within 200-300 nm of the plasma membrane remained unchanged (Figure 12A). Consistent with increased granule docking, acute treatment with IL-1β (10 ng/ml) also increased depolarization-induced exocytosis from dispersed mouse β-cells (Figure 12B) and enhanced synaptosomal-associated protein 25 (SNAP25)/syntaxin-1 SNARE complex formation in INS 832/13 cells (Figure 12C).

Figure 12. Acute treatment with IL-1β increases the number of docked insulin granules. ►

A: Representative electron micrographs of mouse β-cells treated with vehicle or IL-1β (10 ng/ml), as indicated (left), and quantification of the number of granules per micron of plasma membrane (right) (*n* = 51, 50, 56, 55, 51, 51, 57, 55, 52, 50, 57, 55 cells; 3 experiments). Experiments were performed in 2.8 mmol/l glucose, unless otherwise indicated (HG, high glucose = 16.7 mmol/l glucose). Black dashed line indicates plasma membrane. Scale bar represents 500 nm. **B:** Exocytosis responses of single mouse β-cells treated with IL-1β (10 ng/ml) measured as an increase in cell membrane capacitance during a train of membrane depolarization from -70 to 0 mV (left). Average capacitance response to each step-wise depolarization (middle) and total capacitance responses (right) (*n* = 29, 29 cells; 3 experiments); data collection by XD. **C**: Synaptosomal-associated protein 25 (SNAP-25)/syntaxin-1 complex formation in INS 832/13 cells treated with vehicle or IL-1β (10 ng/ml; 30 minutes) assessed by immunoprecipitation (IP) of IgG or syntaxin-1 and immunoblot (IB) of SNAP-25 or syntaxin-1 (left). Quantification by densitometry relative to vehicle-treated control is shown (right) (*n* = 3, 3; 3 experiments). No differences in SNAP-25 expression in input samples were observed (data not shown). *n* values correspond to data points from left to right, respectively. Data are mean ± SEM and were compared with **(A)** 2-way ANOVA followed by Tukey post-test, **(B)** repeated-measures ANOVA followed by Sidak post-test, or **(C)** a 2-tailed Student's *t*-test. *P<0.05, **P<0.01, ***P<0.001 versus vehicle treated control.

Figure 12.


A
mouse β-cells:
0 min.
5 min. HG
15 min. IL-1β
30 min. IL-1β
number of granules / µm of plasma membrane
0 min.
5 min. HG
15 min. IL-1β
30 min. IL-1β
***
***
0 - 0.1
0.1 - 0.2
0.2 - 0.3
granule distance to plasma membrane (µm)
B
mouse β-cells:
0mV
-70mV
+ 2.8mmol/L glucose
vehicle
IL-1β
200pF
cummulative capacitance (fF/pF)
vehicle
IL-1β
depolarization number
total capacitance response (fF/pF)
vehicle
IL-1β
C
INS-1 cells:
IP: syntaxin-1
IB: SNAP-25
IB: syntaxin-1
input:
IB: syntaxin-1
IgG
vehicle
IL-1β
SNAP-25 / syntaxin-1 (% control)
vehicle
IL-1β

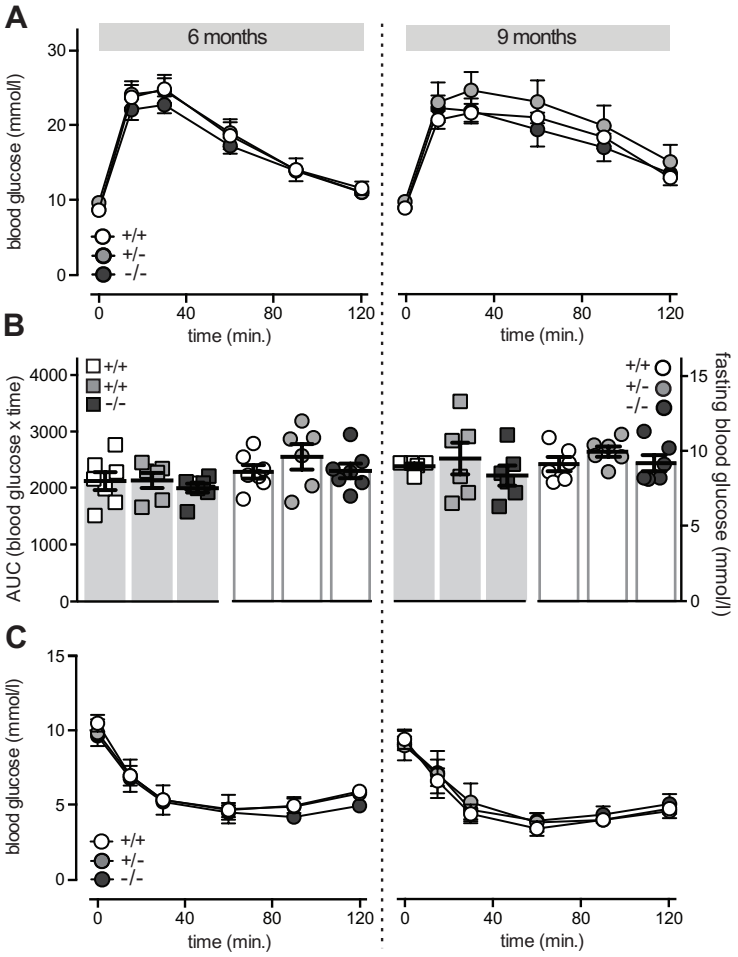
2.4.e IL-1 signaling is not required for the maintenance of glucose tolerance in the absence of metabolic stress.

Consistent with previous reports (García et al., 2006), *Il1r1*-knockout mice were characterized by mature-onset obesity. At 9 months of age, both *Il1r1*-heterozygous (36.4 ± 0.73 g; n=5 mice; $P<0.01$) and knockout mice (35.8 ± 1.23 g; n=5 mice; $P<0.05$) had increased body mass compared with their littermate controls (32.0 ± 1.53g; n=4 mice). Previous reports have observed a mild impairment in glucose tolerance in *Il1r1*-deficient mice (García et al., 2006). In the present study, no significant differences in glucose (Figure 13A) or insulin tolerance (Figure 13B) were observed in unstimulated *Il1r1*-knockout mice compared with littermate controls. These inconsistencies are likely due to differences in experimental protocols, where previous glucose measurements were obtained from non-fasted mice following an intravenous injection of glucose. The experimental protocol in the previous study resulted in a much larger peak in blood glucose (~35 mmol/l glucose) compared with the peak obtained with the present protocol (~25 mmol/l glucose; Figure 13A). Consistent with a role of IL-1 signaling in acute islet compensation to metabolic stress, glucose intolerance was observed in *Il1r1*-deficient mice following an elevated bolus glucose peak (García et al., 2006), but was absent in *Il1r1*-deficient mice following a more moderate glucose load.

Figure 13. *Il1r1*-deficient mice have normal glucose tolerance. ►

A: Blood glucose measurements following intraperitoneal glucose injection of 6 and 9 month wild-type (+/+), heterozygous (+/-), and *Il1r1*-knockout (-/-) mice (*n*=7, 6, 7; *n*=6, 6, 6 mice) following a 3-hour fast. **B:** Area under the curve (AUC) for glucose tolerance tests in **(A)** indicated by squares (*n*=7, 6, 7; *n*=5, 6, 6) and fasting blood glucose concentrations following a 3-hour fast indicated by circles (*n*=7, 6, 7; *n*=6, 6, 6 mice). **C:** Blood glucose measurements following intraperitoneal insulin injections from wild-type (+/+), heterozygous (+/-), and *Il1r1*-knockout mice (-/-) (*n*=6, 5, 6; *n*=7, 7, 8 mice) following a 3-hour fast. Data collection by NS with assistance from CH.

Figure 13.


A
6 months
9 months
blood glucose (mmol/l)
30
20
10
0
+/+
+/-
-/-
0
40
80
120
time (min.)
B
AUC (blood glucose x time)
4000
3000
2000
1000
0
+/+
+/+
-/-
fasting blood glucose (mmol/l)
15
10
5
0
+/+
+/-
-/-
C
blood glucose (mmol/l)
15
10
5
0
+/+
+/-
-/-
time (min.)

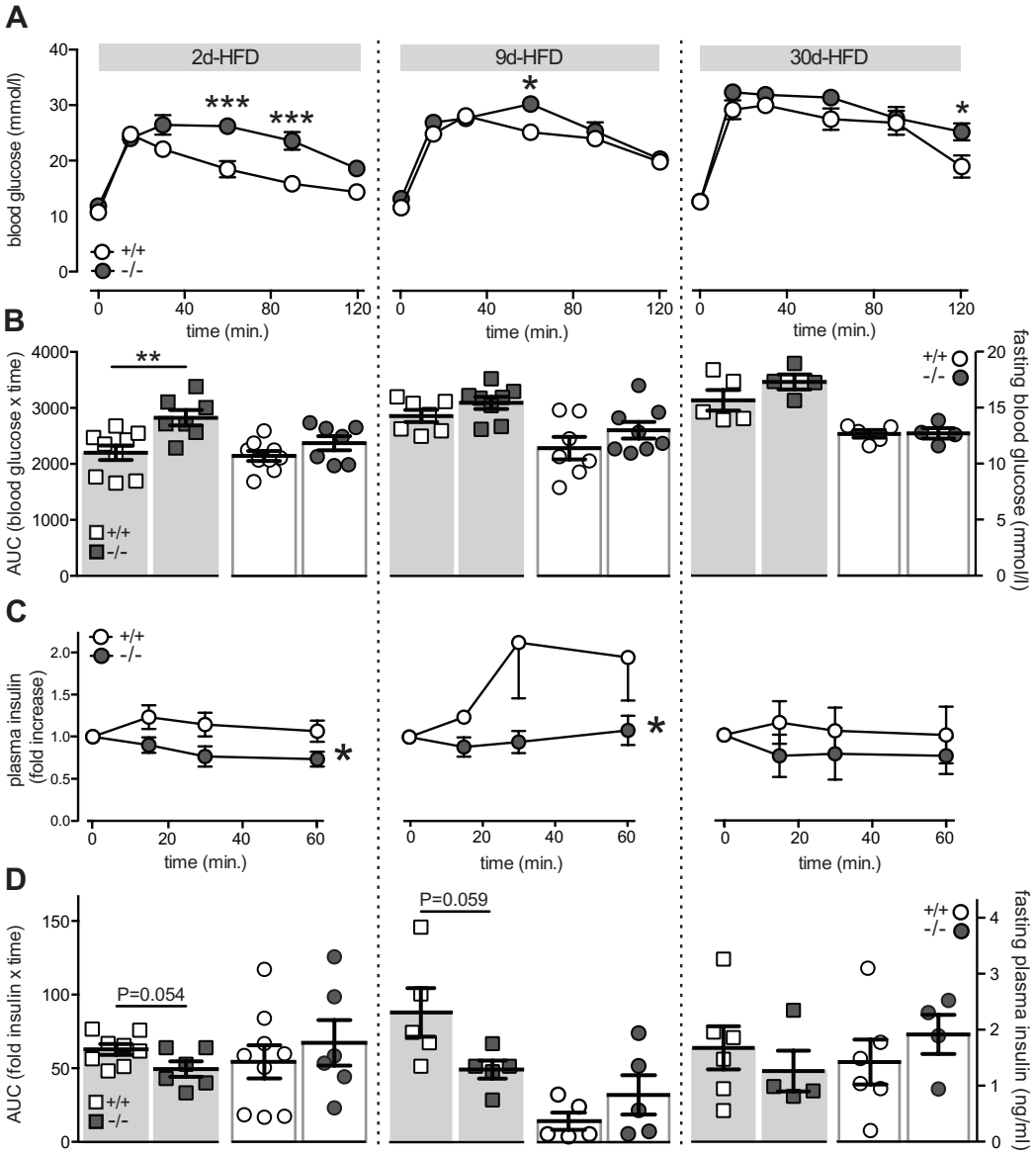
2.4.f *Il1r1*-deficiency impairs glycemic control and reduces in vivo insulin secretory response under conditions of short-term metabolic stress.

β-cell compensation in response to metabolic stress is required for the maintenance of normoglycemia (Kahn et al., 2014; Prentki and Nolan, 2006). In rodents, β-cell compensation upon overnutrition occurs rapidly, with adaptations reported as early as 3 days after HFD (Mosser et al., 2015). Given that this acute adaptation occurs concomitantly with both increases in systemic (Lee et al., 2011) and intra-islet inflammation (Böni-Schnetzler et al., 2009), the role of IL-1R1 signaling in acute islet compensation to metabolic stress was examined. Mice deficient in *Il1r1* (Figure 14A) were fed HFD for 2, 9, or 30 days. *Il1r1*-knockout mice had impaired plasma glucose tolerance following intraperitoneal injection of glucose after 2 days of HFD (Figure 14, A and B, left). Mild glucose intolerance was also observed in knockout mice at 9 (Figure 14, A and B, middle) and 30 (Figure 14, A and B, right) days after HFD. The glucose intolerance induced by *Il1r1* deficiency following 2 (Figure 14, C and D, left) and 9 (Figure 14, C and D, middle) days after HFD was associated with a reduction in the plasma insulin response to glucose, suggestive of impaired β-cell function. Consistent with the acute insulinotropic effect of IL-1β, impaired insulin secretory responses were no longer apparent in *Il1r1*-deficient mice after 30 days of HFD (Figure 14C). No differences in fasting blood glucose (Figure 14B), fasting plasma insulin (Figure 14D), or weight (data not shown) at 2, 9, or 30 days were observed in these 10- to 14-week-old mice.

Figure 14. Impaired glucose tolerance and insulin secretory response in *Il1r1*-knockout mice fed a short-term HFD. ►

Wild-type (+/+) and *Il1r1*-knockout (-/-) mice were fed a HFD for 2, 9, or 30 days. **A:** Blood glucose measurements following intraperitoneal injection of glucose, subsequent to a 3-hour fast, from wild-type and knockout mice (*n*=9, 7; *n*=7, 8; *n*=5, 4 mice). **B:** Area under the curve (AUC) for glucose tolerance tests in **(A)** indicated by squares (*n*=9, 7; *n*=7, 8; *n*=5, 4 mice) and fasting blood glucose concentrations following a 3-hour fast, indicated by circles (*n*=9, 7; *n*=7, 8; *n*=5, 4 mice). **C:** Fold increase in plasma insulin concentrations over values at 0 minutes following intraperitoneal injection of glucose, subsequent to a 3-hour fast, in wild-type and knockout mice (*n*=9, 6; *n*=5, 5; *n*=6, 4 mice). **D:** Area under the curve for plasma insulin in **(C)** indicated by squares (*n*=8, 6; *n*= 5, 5; *n*=6, 4 mice) and fasting plasma insulin concentrations (in ng/ml) following a 3-hour fast indicated by circles (*n*= 9, 6; *n*= 5, 5; *n*=6, 4 mice). *n* values correspond to data points from left to right and to 2-, 9-, and 30-day graphs, respectively. Data collected by NS with assistance from CH. Data are mean ± SEM and were compared with **(B, D)** a 2-tailed Student's *t*-test or **(A, C)** repeated-measures ANOVA followed by **(A)** Sidak post-test. *P<0.05, **P<0.01, ***P<0.001 versus wild-type controls or as indicated.

Figure 14.


A
2d-HFD
9d-HFD
30d-HFD
blood glucose (mmol/l)
time (min.)
+/+
-/-
B
AUC (blood glucose x time)
fasting blood glucose (mmol/l)
C
plasma insulin (fold increase)
D
AUC (fold insulin x time)
P=0.054
P=0.059
fasting plasma insulin (ng/ml)

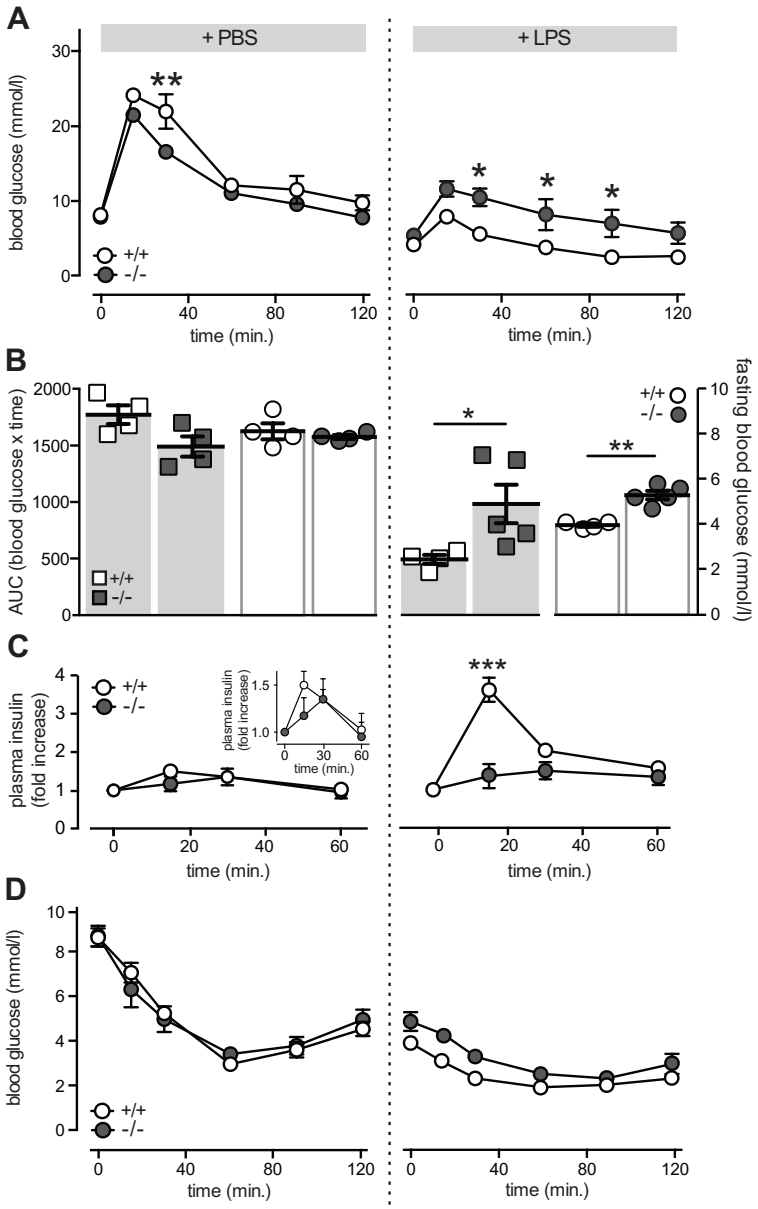
2.4.g LPS-induced potentiation of insulin secretion is mediated by IL-1R1.

A positive role for IL-1 signaling in acute compensation to inflammatory stress was confirmed in mice injected with the pro-inflammatory endotoxin LPS. Consistent with previous reports (Oguri et al., 2002), LPS injection (2 mg/kg) enhanced glucose tolerance in control mice (Figure 15A) by enhancing insulin secretion (Figure 15C). The increase in glucose tolerance following LPS injection was reduced in mice lacking *Il1r1* (Figure 15, A and B). This was not attributable to changes in insulin tolerance (Figure 15D), but rather to enhanced β-cell secretory function, as the insulin secretory response induced by LPS was absent in *Il1r1*-deficient mice (Figure 15B). Despite a blunted LPS-enhanced secretory response, normal glucose tolerance was not restored in *Il1r1*-deficient mice (Figure 15A), indicating the presence of IL-1R1- and β-cell independent effects of acute inflammation on glucose tolerance. Indeed, elevated fasting blood glucose levels in LPS-treated *Il1r1*-knockout mice were not associated with increases in fasting plasma insulin (data not shown). No differences in body weight in these mice were observed (data not shown).

Figure 15. LPS-potentiation of insulin secretion is mediated by IL-1R1. ►

A: Blood glucose measurements following intraperitoneal glucose injections from wild-type (+/+) and *Il1r1*-knockout (-/-) mice treated with LPS (2 mg/kg) or PBS (*n*=4, 4; *n*=4, 5 mice) and fasted for 6 hours. **B**: Area under the curve (AUC) for glucose tolerance tests in **(A)** indicated by squares (*n*=4, 4; *n*=4, 5 mice) fasting blood glucose concentrations in wild-type and knockout mice following a 6-hour fast indicated by circles (*n*=4, 4; *n*=4, 5 mice). **C:** Fold increase in plasma insulin concentrations over values at 0 minutes following intraperitoneal injection of glucose, subsequent to a 6-hour fast and treatment with LPS or PBS in wild-type or knockout mice. Comparison of secretory response in PBS-treated wild-type and knockout mice (inset; *n*=4, 4 mice). **D:** Blood glucose measurements following intraperitoneal insulin injections, subsequent to a 6-hour fast, from wild-type and knockout mice treated with LPS or PBS (*n*=4, 4; *n*=3, 4 mice). *n* values correspond to data points from left to right, respectively. Data collected by NS with assistance from CH. Data are mean ± SEM and were compared with **(A, C, D)** repeated-measures ANOVA followed by Tukey post-test or **(B)** a 2-tailed Student's *t*-test. *P<0.05, **P<0.01, ***P<0.001 versus wild-type controls or as indicated.

Figure 15.


A
+ PBS
+ LPS
blood glucose (mmol/l)
time (min.)
+/+
-/-
B
AUC (blood glucose x time)
fasting blood glucose (mmol/l)
C
plasma insulin (fold increase)
D
blood glucose (mmol/l)

2.5 DISCUSSION

Immunomodulation of metabolism is regarded as an unfavorable consequence of overnutrition that results in metabolic dysfunction (Donath and Shoelson, 2011; Kahn et al., 2014; Lackey and Olefsky, 2015). Inflammation, however, is primarily an acute repair mechanism functioning to preserve homeostasis in response to external stress (Dinarello, 2010). The acute roles of inflammation on metabolic function, particularly in the context of metabolic stress, remain relatively unknown. The aim of this study was to explore roles of the pro-inflammatory cytokine IL-1β and its receptor, IL-1R1, on β-cell function and glucose homeostasis. Here, we provide new insights into the mechanism regulating IL-1β-mediated potentiation of insulin secretion and identify IL-1 signaling as a positive regulator of glycemic control in the acute stages of inflammatory and metabolic stress.

β-cells undergo both structural and functional changes to compensate for the increased insulin demand that is characteristic of obesity (Prentki and Nolan, 2006). Several mechanisms are known to mediated β-cell adaptation, ranging from increased β-cell proliferation (Mosser et al., 2015) and mass (Butler et al., 2003) to enhanced β-cell secretory function (Gonzalez et al., 2013) and sensitivity to insulinotropic stimuli (Nolan et al., 2006). We find that the magnitude of IL-1β potentiation of glucose-stimulated insulin secretion is positively correlated with human donor BMI, where islets from lean human donors have minimal responses to IL-1β. Conversely, IL-1β potentiates glucose-stimulated insulin secretion by almost 2-fold in islets from obese donors, indicating an obesity-associated sensitization of islets to the stimulatory effects of IL-1β. IL-1β levels are elevated in overweight patients (El-Wakkad et al., 2013; Misaki et al., 2010); are increased following acute, post-prandial hyperglycemia (Sage et al., 2012); and are up-regulated in islets

following a 4-day exposure to high glucose or free fatty acids (Böni-Schnetzler et al., 2009; Maedler et al., 2002). A sensitization to the insulinotropic effects of this cytokine may represent a compensatory mechanism to metabolic stress. Indeed, in our hands, while IL-1 signaling was not required for optimal glucose homeostasis under physiological conditions, this emerged as a regulator of glucose tolerance following exposure to acute metabolic stress. This is consistent with findings demonstrating the sensitization of islets from obese Zucker fatty rats to the insulinotropic effects of palmitate and GLP-1 (Nolan et al., 2006)—a phenomenon that is absent in Zucker lean control rats and that promotes the hyperinsulinemia required to compensate for the insulin-resistance characteristic of these animals.

A critical step in the progression to overt diabetes is the maladaptation of β-cells to metabolic stress. When β-cell compensation for insulin resistance ceases, hyperglycemia develops and type 2 diabetes prevails (Kahn et al., 2014; Prentki and Nolan, 2006). Here, we find that the stimulatory potential of IL-1β was lost in islets from obese T2D donors, indicative of impaired or failed β-cell adaptation in these patients. Where β-cells from obese individuals with normal glucose tolerance had a heightened sensitivity to the insulinotropic effects of IL-1β, β-cells from obese T2D donors were unresponsive.

A causal association between IL-1 signaling and islet compensation to acute inflammatory and metabolic stress was identified in the present study. Transcript expression of several inflammatory markers, including *Il1b* and its downstream targets, are up-regulated as early as 3 days in HFD-fed mice (Lee et al., 2011). That the up-regulation of IL-1β contributes to acute islet compensation is supported by observations that *Il1r1*-deficient mice become glucose intolerant more rapidly following short-term HFD feeding compared with littermate controls. The glucose intolerance observed in these animals was attributed to β-cell

dysfunction, as evidenced by an impaired insulin secretory response in *Il1r1*-deficient mice. The role of IL-1 signaling in islet compensation was acute in nature, as the impairment in the in vivo insulin secretory response was no longer observed 30 days after HFD. The role of inflammatory responses in mediating β-cell compensation was further confirmed in an acute model of inflammation. The robust increase in plasma insulin following glucose injection characteristic of LPS administration was absent in mice deficient in *Il1r1*, consistent with the acute insulinotropic effects of IL-1β observed in vitro and with reported correlations between IL-1β concentrations and insulin secretion in vivo (Bissonnette et al., 2015).

While the ability of IL-1β to directly enhance glucose-stimulated insulin secretion from pancreatic islets was reported almost 3 decades ago (Zawalich and Diaz, 1986), the mechanisms underlying this phenomenon remain unclear (Arous et al., 2015; Jeong et al., 2002; McDaniel et al., 1988). Elevated concentrations of IL-1β are known to rapidly enhance insulin secretion in a stimulus-dependent manner (McDaniel et al., 1988; Zawalich and Diaz, 1986) without affecting insulin content (Arous et al., 2015). These findings indicate that the insulinotropic effects of IL-1β are independent of its previously reported effects on proliferation (Maedler et al., 2006) and are not due to unregulated β-cell lysis. That IL-1β potentiates insulin secretion induced by sulfonylureas (Eizirik et al., 1995) or KCl (Arous et al., 2015) demonstrates that the stimulatory effects of this cytokine are, at least in part, independent of its effects on metabolism (Eizirik and Sandler, 1989) and the "amplification pathway" of insulin secretion (Henquin, 2000). While the insulinotropic effects of IL-1β require extracellular Ca^{2+} (Eizirik and Sandler, 1989), IL-1β did not increase $[Ca^{2+}]_i$ or Ca^{2+} currents. Together, these data confirm that IL-1β is able to potentiate insulin secretion by acting at sites downstream of Ca^{2+} entry (Eizirik et al., 1995; Helqvist et al., 1989; Welsh et al., 1989). Although this indicates that the insulinotropic effects of IL-1β are

downstream of $[Ca^{2+}]_i$, current findings cannot preclude the possibility that IL-1β may also potentiate glucose-stimulated insulin secretion by acting on parallel pathways that are upstream of $[Ca^{2+}]_i$.

A modest depolymerization of cortical F-actin following exposure to IL-1β in both mouse and human β-cells was observed, suggesting that IL-1β potentiates stimulus-induced insulin secretion in part by inducing the depolymerization of F-actin. IL-1β, however, also enhanced glucose-stimulated insulin secretion in the presence of complete F-actin depolymerization, demonstrating the ability of IL-1β to potentiate insulin secretion through an F-actin depolymerization-dependent pathway. Time differences in responses to IL-1β-induced potentiation of cortical F-actin depolymerization between human and mouse islets were observed. While these differences may be due to the use of recombinant human—rather than murine—IL-1β, that mechanistic differences underlie variances in cortical F-actin depolymerization is evidenced by findings that recombinant human IL-1β induces effects on granule trafficking and exocytosis within 30 minutes in mouse β-cells. Acute treatment with IL-1β was found to enhance granule density within 200 nm of the plasma membrane and increase SNARE complex formation in vitro. Consistent with IL-1β-induced stimulation of granule protein exocytosis from human neutrophils (Smith et al., 1986), these morphological and molecular observations of granule docking resulted in functional effects, where IL-1β was found to acutely enhance β-cell exocytosis.

Recently, the IL-1β-induced remodeling of FA was associated with the insulinotropic actions of IL-1β (Arous et al., 2015). FA proteins function to integrate the extracellular matrix with cytoskeletal proteins and are known regulators of β-cell actin remodeling and insulin secretion (Arous et al., 2015; Cai et al., 2012; Rondas et al., 2012). In addition to their

roles in cytoskeletal rearrangement, FA proteins have been implicated as regulators of SNARE complex formation with F-actin (Rondas et al., 2012). The effects of IL-1β on both actin and exocytotic complex formation are supportive of FA proteins as regulators of the insulinotropic effect of IL-1β. However, latrunculin B inhibits the glucose-induced activation of the FA proteins PTK2 and PXN (Rondas et al., 2012). That IL-1β potentiates insulin secretion in the presence of latrunculin B suggests an FA-independent pathway of IL-1β potentiation. While the molecular mechanisms underlying the insulinotropic effect of IL-1β remain unclear, the present study demonstrates that potentiation of glucose-stimulated insulin secretion by IL-1β is mediated by distal effects on the stimulus-secretion coupling pathway, resulting in increased insulin granule docking and exocytosis.

2.6 STUDY LIMITATIONS

IL-1 signaling is required for the maintenance of glucose homeostasis in response to acute metabolic stress. Although evidence presented in the current study suggests that this is mediated by a direct action of IL-1β on β-cell secretory function, β-cell-independent effects cannot be excluded due to the use of a whole-body knockout model. Consistent with the central hypophagic role of IL-1β (McCarthy et al., 1985), weight and feeding behavior are increased in *Il1r1*-deficient mice around 6 months of age (García et al., 2006). However, that food intake and body mass are similar between 4-month old wild-type and *Il1r1*-knockout mice (García et al., 2006), and that no differences in weight were observed in 10 to 14 weeks old mice of the current study, suggests that central effects of IL-1β on feeding behavior are unlikely responsible for the glucose intolerance observed.

IL-1 signaling has also been implicated in the regulation of insulin sensitivity under conditions of chronic metabolic stress (García et al., 2006; McGillicuddy et al., 2013). While a rise in insulin resistance could result in the glucose intolerance described, insulin action in 6-month old chow-fed *Il1r1*-knockout mice is similar to wild-type littermates (McGillicuddy et al., 2013; Figure 13C). Furthermore, changes in insulin sensitivity were not associated with effects of IL-1 signaling on glucose tolerance in LPS-treated animals, and preliminary evidence suggests this is also true in the context of acute metabolic stress (Figure 16). Nevertheless, the use of a β-cell-specific *Il1r1*-knockout model, generated through breeding of the newly-characterized, commercially available $Il1r^{loxP/loxP}$ (Robson et al., 2016; The Jackson Laboratory) and $Ins1^{cre}$ (The Jackson Laboratory; Thorens et al., 2015) mice, would assist in confirming that the compensatory effects of IL-1 signaling on glucose homeostasis are mediated by the pancreatic β-cells.

β-cell proliferation is rapidly increased upon exposure to metabolic stress, with proliferation markers present within 3 days of HFD (Mosser et al., 2015). IL-1β regulates β-cell proliferation in vivo (Maedler et al., 2006), where an IL-1β-induced up-regulation of β-cell replication may contribute to the enhanced secretory response observed 2-days post-HFD in wild-type mice. However, a detectable increase in β-cell mass is not apparent until week 3 of HFD feeding (Mosser et al., 2015), at which time secretory function of *Il1r1*-deficient mice is similar to controls.

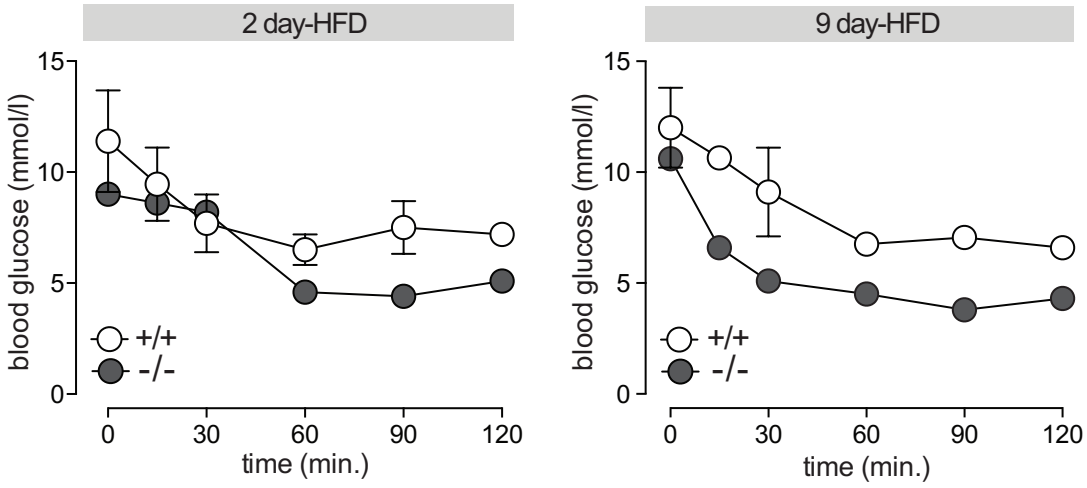
Quantification of IL-1β concentrations in serum of mice exposed to acute HFD or LPS was not assessed in the present study. Acute HFD induces transcription of IL-1β-specific target genes in murine adipose tissue within 3 days (Lee et al., 2011), indicating that, in addition to the induction of systemic inflammation, acute exposure to HFD enhances expression of IL-1β. Consistent with these findings, *IL1B* is up-regulated in islets exposed to elevated free fatty acid concentrations (Böni-Schnetzler et al., 2009). Furthermore, that *Il1r1*-deficiency resulted in pronounced effects upon exposure to HFD indicates that enhanced sensitivity to, or increased production of, IL-1β is induced by acute metabolic stress.

The current study is indicative of a T2D islet maladaptation to the insulinotropic effects of IL-1β. Increasing the sample size of obese T2D donors would undoubtedly add certainty to the conclusions of the study. Unfortunately, access to human islet tissue (Kulkarni and Stewart, 2014), particularly to that of obese T2D human donors is limiting, with IsletCore distribution averaging 1 obese T2D donor every 7 months. While the absence of IL-1β action should be confirmed as human donor tissue becomes available, that IL-1β was unable to potentiate insulin secretion in all three T2D donors examined was, in itself, significant.

Figure 16. Acute metabolic stress does not appear to alter insulin sensitivity in *Il1r1*-deficient mice. ►

Blood glucose measurements following intraperitoneal insulin injections following a 3-hour fast from wild-type (+/+) or *Il1r1*-knockout mice (-/-) fed a HFD for 2 days (*n*=2, 1 mice; left) or 9 days (*n*=2, 1 mice; right). *n* values correspond to wild-type and *Il1r1*-knockout mice, respectively.

Figure 16.


2 day-HFD
9 day-HFD
blood glucose (mmol/l)
15
10
5
0
+/+
-/-
0
30
60
90
120
time (min.)

2.7 CONCLUDING REMARKS

Evidence presented in the current chapter proposes that IL-1 signaling contributes to islet compensation in response to acute metabolic stress by enhancing secretory function subsequent to increased granule trafficking and SNARE complex formation. A compensatory role for IL-1 signaling is further supported by findings that islets from obese donors are sensitized to the stimulatory effects of IL-1β, whereas the insulinotropic effects of this cytokine are absent in obese T2D islets. This study highlights a positive role of IL-1 signaling in glucose homeostasis by mediating an enhancement of glucose-stimulated insulin secretion early in response to metabolic stress in mice and in islets from obese, but non-diabetic humans.

While the present chapter establishes a beneficial role for acute IL-1 signaling in vivo, it does not diminish the well-characterized, detrimental actions of chronic IL-1 signaling. Rather, it highlights the importance of understanding the temporal, metabolic, and molecular regulation of these processes. Implication of both SUMOylation (Ferdaoussi et al., 2015) and IL-1 signaling in T2D-associated β-cell dysfunction is suggestive of a potential interaction between these two pathways. Consequently, the following chapter investigates the role of SUMOylation as a regulator of IL-1 signaling and as a mediator of β-cell viability.

CHAPTER 3.

SUMOylation protects against IL-1β-induced apoptosis.

The following chapter is adapted from work published in the *American Journal of Physiology Endocrinology and Metabolism.* It is reprinted with permission of the American Physiological Society under the following citation:

Hajmrle, C., Ferdaoussi, M., Plummer, G., Spigelman, A.F., Lai, K., Manning Fox, J.E., and MacDonald, P.E. (2014). SUMOylation protects against IL-1β-induced apoptosis in INS-1 832/13 cells and human islets. Am. J. Physiol. Endocrinol. Metab. *307*, E664-E673.

Co-author contributions to the figures presented are acknowledged in the figure legends by co-author initials.

3.1 ABSTRACT

While increased SUMOylation reduces β-cell exocytosis, insulin secretion, and responsiveness to GLP-1, the impact of SUMOylation on islet cell survival is unknown. In the present work, mouse islets, INS 832/13 cells, or human islets were transduced with adenoviruses targeted to increase either SENP1 or SUMO1 or were transfected with siRNA duplexes to knockdown SENP1. Surprisingly, up-regulation of SENP1 reduces insulin secretion and impairs $[Ca^{2+}]_i$ handling. Secretory dysfunction induced by SENP1 occurs secondary to a SENP1-mediated induction of cell death. Indeed, secretory impairment induced by SENP1 is reduced when two mediators of β-cell death, iNOS and NFκB, are pharmacologically inhibited. Conversely, enhanced SUMOylation protects against IL-1β-induced apoptosis. This is associated with reduced iNOS expression, cleavage of caspase 3, and nuclear translocation of NFκB. Taken together, these findings identify SUMO1 as a novel anti-apoptotic protein in pancreatic islets and suggest that reduced viability accounts for impaired islet function following up-regulation of SENP1.

3.2 INTRODUCTION

SUMO peptides are involved in the post-translational modification of numerous cellular proteins (Gareau and Lima, 2010; Geiss-Friedlander and Melchior, 2007; Kamitani et al., 1997). SUMO modification, known as SUMOylation, occurs through a cascade of well-characterized enzymatic events that rely on the E2 conjugating enzyme UBC9 (Gareau and Lima, 2010; Geiss-Friedlander and Melchior, 2007; Gong et al., 1997). SUMOylation is made reversible by SENP proteases, which remove SUMO from target proteins (Yeh, 2009). Due to the large number of SUMOylatable targets, the cellular outcomes of SUMO modification are diverse, ranging from control of DNA repair to regulation of transcription factors and plasma membrane proteins (Dai et al., 2009; 2011; Geiss-Friedlander and Melchior, 2007; Kishi et al., 2003; Okura et al., 1996; Rajan et al., 2012; Shao and Cobb, 2009).

SUMO1 modification has a negative impact on β-cell secretory function (Manning Fox et al., 2012). Upregulation of SUMO1 decreases insulin gene expression (Kishi et al., 2003; Shao and Cobb, 2009), GLP-1 receptor signaling (Rajan et al., 2012), and both glucose- and exendin-4-stimulated insulin secretion (Dai et al., 2011; Rajan et al., 2012) suggesting that deSUMOylation may improve secretory function. Indeed, knockdown of the gene encoding the SUMOylating enzyme UBC9 enhances exendin-4-stimulated insulin secretion (Rajan et al., 2012) and upregulation of the deSUMOylating enzyme SENP1 increases exocytosis from rodent β-cells at low glucose (Dai et al., 2011; Vergari et al., 2012). Whether SENP1 elevates insulin secretion above that stimulated by glucose is unclear, as SENP1 does not increase β-cell exocytosis above the levels seen with high glucose (Dai et al., 2011). Thus, while increased SUMOylation results in secretory impairment (Dai et al.,

2011; Rajan et al., 2012; Vergari et al., 2012), the effect of the deSUMOylating enzyme SENP1 remains unknown.

SUMOylation is implicated in the regulation of iNOS expression (Akar and Feinstein, 2009; Pascual et al., 2005). While the extent of iNOS involvement in mediating β-cell cytotoxicity is debated, up-regulation of iNOS expression occurs concomitantly with activation of pro-apoptotic pathways involved in β-cell death and dysfunction. Whether SENP1 enhances β-cell death remains unknown. We sought, therefore, to examine the role of SENP1 on secretory function and survival. If deSUMOylation reduces β-cell viability, any potential stimulatory effects of SENP1 on insulin secretion may be overshadowed.

In the present work, we demonstrate that SENP1 over-expression induces secretory dysfunction in islets. Rather than inhibiting secretion at the exocytotic site, as is observed when SUMOylation is increased, SENP1 impairs intracellular Ca^{2+} handling following the induction of apoptotic signaling by deSUMOylation. SENP1 enhances cell death in both an insulin secreting cell line (INS 832/13) and in human islet cells, where the detrimental effect of SENP1 on secretory function is no longer observed when iNOS or NFκB are pharmacologically inhibited. Conversely, enhanced SUMOylation, induced either by over-expression of SUMO1 or knockdown of SENP1, reduces stimulus-induced cell death in INS 832/13 and human islet cells. This is associated with decreased iNOS expression, cleavage of caspase 3, and nuclear translocation of NFκB. Thus, SUMOylation plays both positive and negative roles in pancreatic islets: while SUMOylation impairs insulin exocytosis (Dai et al., 2011), it also protects against apoptosis. Understanding the spatial and temporal regulation of SUMOylation may allow targeted modulation of islet survival and function.

3.3 MATERIALS AND METHODS

3.3.a Cells and cell culture.

Islets from male C57/BL6 mice were isolated by collagenase digestion and cultured in RPMI 1640 containing 11.1 mmol/l glucose with 10% FBS and 100 units/ml of penicillin/streptomycin. Human islets from 8 healthy donors (age 61 ± 3.6 years) from the Clinical Islet Laboratory at the University of Alberta and the Alberta Diabetes Institute IsletCore were cultured in low-glucose (5.5 mmol/l) DMEM with L-glutamine, 110 mg/l sodium pyruvate, 10% FBS, and 100 units/ml penicillin/streptomycin. INS 832/13 cells, a rat insulinoma cell line from Dr. C. Newgard (Duke University), were cultured in RPMI 1640 containing 11.1 mmol/l glucose with 10% FBS, 10 mmol/l HEPES, 0.29 mg/ml L-glutamine, 1 mmol/l sodium pyruvate, 50 μmol/l β-mercaptoethanol, and 100 units/ml penicillin/streptomycin. Islets and INS 832/13 cells were cultured at 37°C and 5% CO_2. The animal care and use committee and the Human Research Ethics Board at the University of Alberta approved all studies.

3.3.b Adenoviruses, constructs, and treatments.

Recombinant adenoviruses producing green fluorescent protein (GFP) alone (Ad-GFP), or together with SUMO1 (Ad-SUMO1) or SENP1 (Ad-SENP1), were created using pAdtrackCMV and the AdEasy system (www.coloncancer.org) as described (Dai et al., 2011; Vergari et al., 2012). Equivalent, commercially produced adenoviruses, Ad-GFP, Ad-GFP-SENP1, Ad-GFP-SUMO1 (Welgen Inc., Worcester, MA) were also utilized at a titer of ~$6.7x10^2$ viral particles per INS 832/13 cell, $1x10^4$ viral particles per dispersed human islet

cell, and $2x10^7$ viral particles per mouse or human islet. Whole islets or cells were infected for 14–18 h. Experiments were performed 40–44 h post-infection.

INS 832/13 cells were transfected for 40–44 h with human SUMO1-GFP in the pEGFP-C1 vector (a gift from Dr. Steven Ogg, University of Alberta) or the pIRES-EGFP control vector (Clontech, Palo Alto, CA) using Lipofectamine 2000 (Life Technologies, Burlington, ON). INS 832/13 cells were transfected for 48 h (cleaved caspase 3) or 66 h (*Nos2* mRNA) with rat siSENP1 and siScram control duplexes (Applied Biosystems, Burlington, ON) using Dharmafect (Thermo Scientific, Ottawa, ON).

Following infection or transfection, culture media was changed to fresh medium containing glucose and/or human recombinant IL-1β (Sigma, Oakville, ON), as indicated. Where pharmacological inhibitors were used, ammonium pyrrolidinedithiocarbamate (PDTC; Sigma) or L-N^6-(1-iminoethyl)lysine dihydrochloride (L-NIL; Sigma) were added to culture media simultaneously with adenoviruses for infection. Following infection period, medium was replaced with fresh media containing PDTC or L-NIL.

3.3.c Insulin and proinsulin secretion assay.

Measurements were performed at 37°C in KRB containing (in mmol/l): 135 NaCl, 3.6 KCl, 5 $NaHCO_3$, 1.5 $CaCl_2$, 0.5 $MgCl_2$, 0.5 NaH_2PO_4, 10 HEPES, and 0.1% BSA (pH 7.4). Intact mouse or human islets were pre-incubated for 2 h in either 2.8 or 1.0 mmol/l glucose KRB, respectively. Islet perifusion was performed with KRB containing 20 mmol/l KCl and 2.8 mmol/l glucose at a flow rate of 250 μl/min. Solution was collected every 2 minutes. Osmolarity in the high-KCl solution was corrected by reducing NaCl. For static secretion, islets were transferred to fresh KRB solution containing 2.8 or 1.0 mmol/l glucose for 1 h

followed by incubation for 1 h in 16.7 mmol/l glucose-KRB. Supernatant fractions were collected and islets were lysed in buffer containing 1.5% concentrated hydrochloric acid, 23.5% acetic acid, and 75% ethanol for assay of protein and insulin content. Samples were stored at -20°C and assayed for insulin via Insulin Detection Kits (Meso Scale Discovery, Rockville, MD), for proinsulin via a mouse Proinsulin ELISA (Alpco Diagnostics, Salem, NH), and for protein using a Micro BCA Protein Assay Kit (Thermo Scientific, Rockford, IL).

3.3.d $[Ca^{2+}]_i$ measurements.

Intact mouse islets were incubated for 45 minutes with 10 μmol/l Fura Red-AM (Life Technologies, Burlington, ON) and 0.08% pluronic acid (Life Technologies) in solution containing (in mmol/l): 130 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 5 $NaHCO_3$, and 10 HEPES (pH 7.4). Islets were imaged in 0.5 mmol/l glucose at 37°C with constant bath perfusion. Glucose was increased to 11 mmol/l, as indicated. Imaging was performed with a Stallion imaging system (Olympus Canada, Richmond Hill, ON) and Ratio Cam software (Metamorph, Molecular Devices, Sunnyvale, CA). Excitation was at 440 and 490 nm and emission was collected using a 660/50 nm bandpass filter for ratiometric imaging. The glucose-stimulated $[Ca^{2+}]_i$ (GSCa) response was calculated as the fold-increase in the Fura-Red ratio following 11 mmol/l glucose versus the ratio at 0.5 mmol/l glucose. The standard deviation between 650 to 750 s of the response was taken as an indicator of oscillatory activity.

3.3.e Cell death measurements.

Cell death assays were performed on INS 832/13 or dispersed human islet cells using

the *In Situ* Cell Death Detection Kit, TMR Red, (Roche, Mannheim, Germany), using TUNEL technology, according to the manufacturer's directions. Images were obtained using a Zeiss AxioObserver Z1 with a Zeiss-Colibri light source at 488 and 594 nm, a 40X/1.3 NA lens, and an AxioCam HRm camera. Images were acquired in Axiovision 4.8 software (Carl Zeiss MicroImaging GmbH, Göttingen, Germany).

3.3.f Immunoblotting.

INS 832/13 cells or mouse islets were lysed in buffer containing (in mmol/l): 20 Tris-HCl (pH 7.5), 150 NaCl, 1 EGTA, 1 EDTA, 25 *N*-ethylmaleimide, 1% triton X-100, and protease inhibitor cocktail (Set V; Millipore, Billerica, MA). Whole cell lysates were separated using SDS-PAGE, transferred to polyvinylidene difluoride membranes (Millipore), probed with primary antibodies [anti-cleaved caspase 3 (1:1000), Cell Signaling Technology, Danvers, MA; anti-SENP1 (1:1000), Cell Signaling Technology; anti-SUMO1 (1:1000), Santa Cruz Biotechnology, Santa Cruz, CA; anti-β-tubulin (1:30,000), Sigma, Oakville, ON; anti-β-actin (1:15,000), Santa Cruz Biotechnology; anti-iNOS (1:10,000), BD Biosciences, Mississauga, ON)], and detected with appropriate peroxidase-conjugated secondary antibodies (Amersham, Baie d'Urfe, PQ). Densitometry was analyzed with ImageJ software (http://imagej.nih.gov/ij/).

3.3.g Immunohistochemistry and immunocytochemistry.

Intact human islets were fixed in 10% Shandon Zinc Formal-Fixx (Thermo Scientific, Rockford, IL) and embedded in 2% wt/vol low melting point agarose. Sections (3 μm) were rehydrated and antigen unmasking was performed. Slides were blocked in buffer containing

5% normal goat serum and 0.3% triton X-100 in PBS. All antibodies were diluted in a buffer containing 1% BSA and 0.3% triton X-100 in PBS. Cleaved caspase 3 was detected using anti-cleaved caspase 3 (1:200; Cell Signaling Technologies, Danvers, MA) followed by anti-rabbit AlexaFluor 594 (Molecular Probes, Eugene, OR) and GFP was detected using anti-GFP (1:500; from Dr. Luc Berthiaume, www.eusera.com) followed by anti-mouse AlexaFluor 488 (Molecular Probes).

INS 832/13 cells were washed in PBS, fixed in 10% Shandon Zinc Formal-Fixx (Thermo Scientific, Rockford, IL), quenched with 50 mmol/l NH_4Cl, permeabilized with 0.1% triton X-100, and blocked for 30 min in 20% donkey serum (Sigma, Oakville, ON) in PBS. All antibodies were diluted in 2% donkey serum in PBS. NFκB was detected using anti-p65 (1:200; Santa Cruz Biotechnology, Santa Cruz, CA) followed by anti-rabbit AlexaFluor 594 (Molecular Probes, Eugene, OR). Nuclei were DAPI-labeled using ProLong Gold Antifade with DAPI (Molecular Probes).

Visualization was on a Zeiss AxioObserver Z1 with a Zeiss-Colibri light source at 488 and 594 nm, a 40X/1.3 NA lens, and an AxioCam HRm camera. Images were acquired and analyzed in Axiovision 4.8 software (Carl Zeiss MicroImaging GmbH, Göttingen, Germany).

3.3.h Quantitative PCR.

RNA was extracted using TRIzol Reagent (Life Technologies, Burlington, ON). Real-time quantitative PCR assays were carried out on the 7900HT Fast Real-Time PCR system (Applied Biosystems, Burlington, ON) using Fast SYBR Green Master Mix (Applied Biosystems) as the amplification system with 300 nmol/l primers and 1-µl template in 20-µl

PCR volume and annealing temperature of 60°C. Primers were: rat CHOP forward 5'-GGAGGTCCTGTCCTCAGATG-3', reverse 5'-AGGTGCTTGTGACCTCTGCT-3'; rat ATF4 forward 5'-GTTGGTCAGTGCCTCAGACA-3', reverse 5'-CATTCGAAACAGAGCATCG-3'; rat iNOS forward 5′-GGGAGCCAGAGCAGTACAAG-3′, reverse 5′-GGCTGGACTTC-TCACTCTGC-3'; rat β-actin forward 5′-TGAAGTGTGACGTTGACATCC-3′, reverse 5′-ACAGTGAGGCCAGGATAGAGC-3′.

3.3.i Nitrite Measurement.

Nitrite accumulation in culture medium was determined by use of Greiss reagent (Sigma), where 50 µl of culture medium was mixed and incubated with 50 µl of Griess reagent for 15 minutes at room temperature. Nitrite concentrations were quantified by measuring absorbance at 540 nm. Dilutions of sodium nitrite were used as standards.

3.3.j Statistics.

Data were compared by unpaired *t*-test or by two-way ANOVA followed by a post hoc *t*-test using the Fisher LSD or Tukey HSD method. Outliers were identified and removed using Grubb's test for outliers. Data are expressed as means ± SEM, where $P<0.05$ is considered significant.

3.4 RESULTS

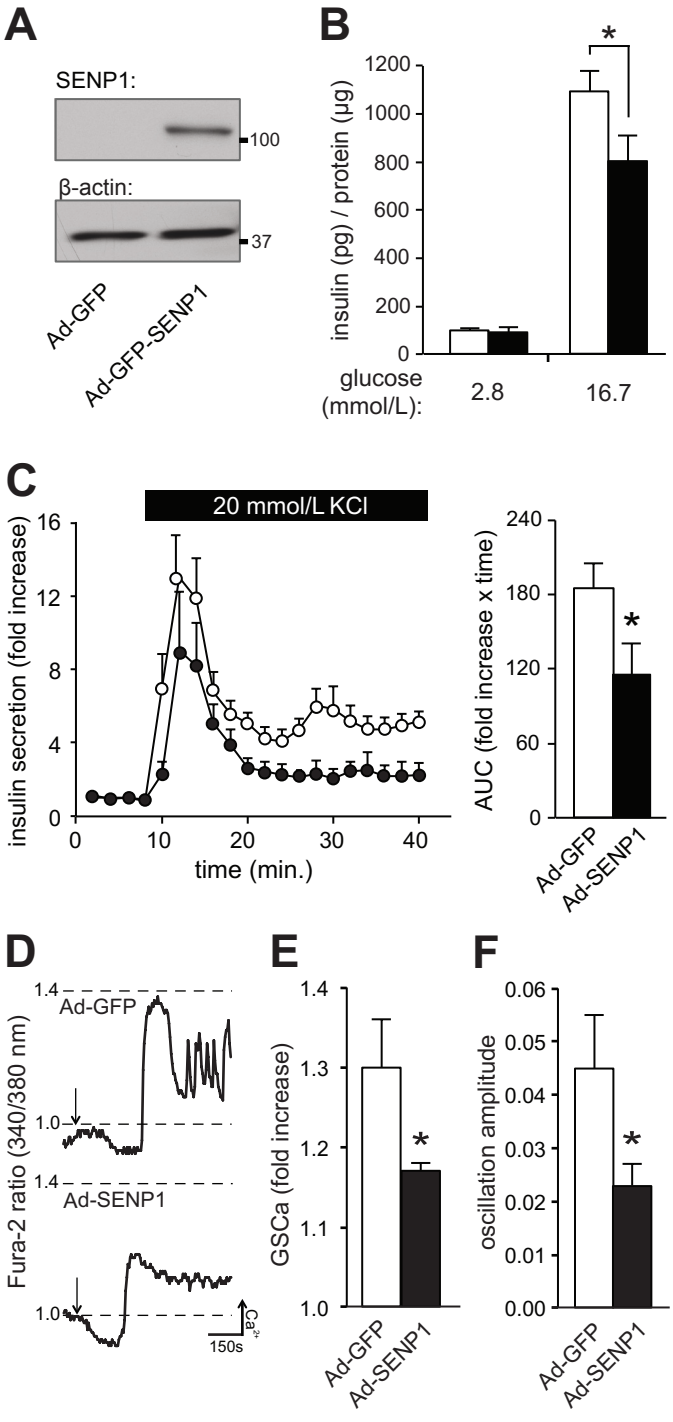
3.4.a SENP1 induces secretory dysfunction in pancreatic islets.

We have previously reported that SENP1 increases depolarization-induced exocytosis from isolated β-cells (Dai et al., 2011; Vergari et al., 2012) suggesting that deSUMOylation may increase insulin secretion. Surprisingly, we found that over-expression of SENP1 (Figure 17A) reduced insulin secretion from mouse islets stimulated by glucose (*n*=8; Figure 17B) or KCl (*n*=3; Figure 17C). Reduced insulin content (71.80 ± 8.64 and 56.73 ± 9.48 ng/μg protein in Ad-GFP- and Ad-GFP-SENP1-infected islets, respectively) could not entirely account for the reduced insulin secretion observed, as there were no significant differences between the groups (*n*=15). To understand the underlying mechanism, whole-islet $[Ca^{2+}]_i$ responses were assessed. Upregulation of SENP1 reduced glucose-stimulated $[Ca^{2+}]_i$ responses (*n*=7; Figure 17E) and amplitude of $[Ca^{2+}]_i$ oscillations (*n*=7; Figure 17F) suggesting that, unlike SUMO1-induced secretory dysfunction, the inhibitory effect of SENP1 on insulin secretion is not downstream of $[Ca^{2+}]_i$ entry. No differences in basal $[Ca^{2+}]_i$ were observed (data not shown).

Figure 17. SENP1 induces secretory dysfunction in islets. ►

A: Exogenous SENP1 tagged to GFP in mouse islets and the loading control β-actin. **B:** Insulin secretion normalized to protein content from mouse islets transduced with Ad-GFP (open bars) or Ad-GFP-SENP1 (filled bars); data collection by CH and AFS. **C:** KCl-stimulated insulin secretion from mouse islets transduced with Ad-GFP (open circles) or Ad-SENP1 (filled circles) normalized to baseline insulin secretion. Area under the curve (AUC) of the secretory response is shown (right); data collection by JMF. **D:** Responses in $[Ca^{2+}]_i$ from mouse islets transduced with Ad-GFP or Ad-SENP1 following glucose-stimulation (arrow). **E:** Glucose-stimulated $[Ca^{2+}]_i$ (GSCa) responses. **F:** Amplitude of $[Ca^{2+}]_i$ oscillations in mouse pancreatic islets. *P<0.05 versus Ad-GFP or as indicated.

Figure 17.


A
SENP1:
100
β-actin:
37
Ad-GFP
Ad-GFP-SENP1
B
insulin (pg) / protein (μg)
1200
1000
800
600
400
200
0
*
glucose (mmol/L):
2.8
16.7
C
20 mmol/L KCl
insulin secretion (fold increase)
16
12
8
4
0
0
10
20
30
40
time (min.)
AUC (fold increase x time)
240
180
120
60
0
*
Ad-GFP
Ad-SENP1
D
Ad-GFP
Ad-SENP1
Fura-2 ratio (340/380 nm)
1.4
1.0
150s
Ca2+
E
GSCa (fold increase)
1.4
1.3
1.2
1.1
1.0
*
Ad-GFP
Ad-SENP1
F
oscillation amplitude
0.06
0.05
0.04
0.03
0.02
0.01
0.00
*
Ad-GFP
Ad-SENP1

3.4.b SENP1 induces cell death.

While the presence of abnormal glucose-stimulated islet $[Ca^{2+}]_i$ oscillations are indicative of reduced β-cell viability (Carter et al., 2009; Dula et al., 2010; Jahanshahi et al., 2009), deSUMOylation is cytoprotective in other cell types (Feligioni et al., 2011; Li et al., 2008). The effects of SENP1 over-production on β-cell death, therefore, were examined. Upregulation of SENP1 (Figure 18A) increased cell death more than 6-fold (*n*=5 experiments (2572 cells); Figure 18B). SENP1 also enhanced cell death in the presence of the pro-inflammatory cytokine IL-1β (*n*=5 experiments (2572 cells); Figure 18B). This is due, at least in part, to an increase in apoptosis, as levels of the apoptotic marker, cleaved caspase 3, were also increased by SENP1 in the absence and presence of IL-1β (*n*=3; Figure 18C).

The ability of SENP1 to induce apoptosis in human islets was also assessed. Cleaved caspase 3 was measured by immunohistochemistry in human islets infected with Ad-SENP1. Infected cells were identified by the presence of GFP. To induce apoptosis, human islets were treated with a combination of IL-1β (10 ng/ml) and high glucose (25 mmol/l) for 48 hours, as treatment with IL-1β alone was unable to increase cleavage of caspase 3 at 48 hours in our hands (data not shown). Upregulation of SENP1 enhanced the apoptotic response to IL-1β and high glucose in all human donors examined (*n*=3 donors (1518 cells); Figure 18D). Consistent with its ability to sensitize human islet cells to IL-1β and high glucose-induced cell death, SENP1 also sensitized mouse islets to the cytotoxic effects of IL-1β and high glucose on β-cell dysfunction. SENP1 over-expression in the presence of a combination of IL-1β (10 ng/ml) and high glucose (25 mmol/l) for 24 hours reduced the $[Ca^{2+}]_i$ response to glucose in mouse islets (Figure 19).

Figure 18. SENP1 induces cell death. ►

A: Exogenous SENP1 tagged to GFP in INS 832/13 cells and the loading control β-actin. **B:** INS 832/13 cells transduced with Ad-GFP (open bars) or Ad-GFP-SENP1 (filled bars) treated with IL-1β for 24 h and stained for TUNEL (red) and nuclei (DAPI, blue). Representative images (left) and quantification of GFP+ and TUNEL+ cells (arrows) as a percentage of total GFP+ cells (right) are shown. Scale bars represent 15 microns. **C:** Immunoblotting for cleaved caspase 3 (CC3) and β-tubulin (left) from INS 832/13 cells transduced with Ad-GFP (open bars) or Ad-GFP-SENP1 (filled bars) and treated with IL-1β for 24 h. Quantification by densitometry relative to Ad-GFP group (right); data collection by KL under supervision of CH. **D:** Human islets transduced with Ad-GFP (white circles) or Ad-SENP1 (open circles) were treated, as indicated, for 48 h. Islets sections immunostained for GFP (green), CC3 (red), and nuclei (DAPI; blue). Representative images (left) and GFP+ CC3+ cells (arrows) as a percentage total GFP+ cells (right) are shown. Scale bars represent 10 microns. *$P<0.05$, **$P<0.01$.

Figure 18.

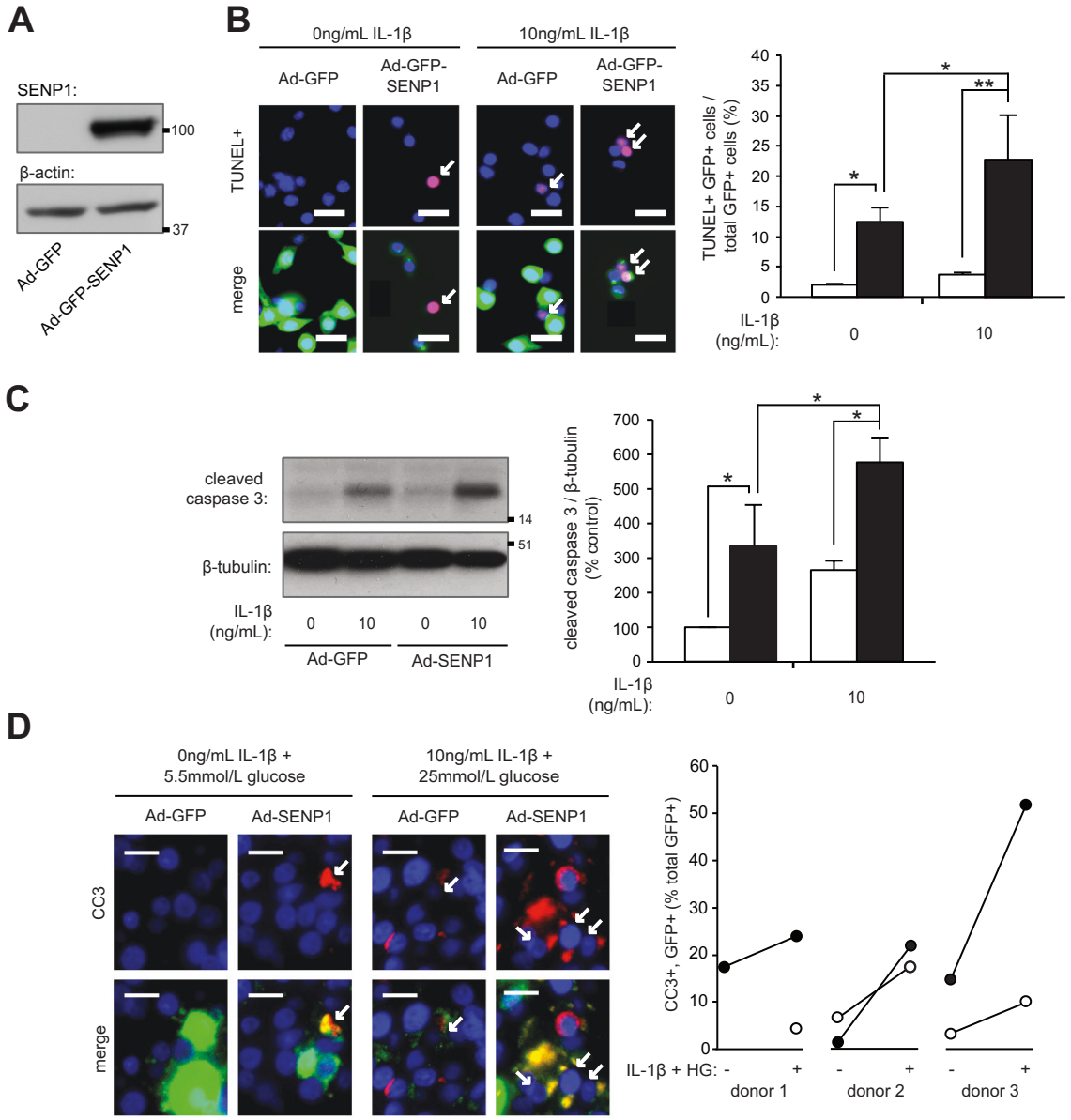
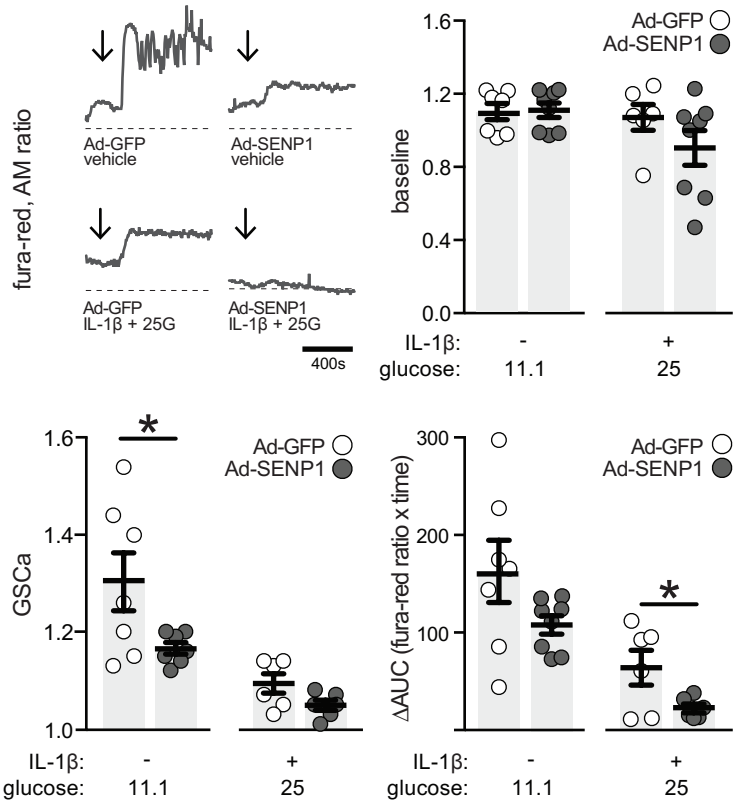

A
SENP1:
100
β-actin:
37
Ad-GFP
Ad-GFP-SENP1
B
0ng/mL IL-1β
10ng/mL IL-1β
Ad-GFP
Ad-GFP-SENP1
Ad-GFP
Ad-GFP-SENP1
TUNEL+
merge
TUNEL+ GFP+ cells / total GFP+ cells (%)
IL-1β (ng/mL):
0
10
C
cleaved caspase 3:
β-tubulin:
14
51
IL-1β (ng/mL):
0
10
0
10
Ad-GFP
Ad-SENP1
cleaved caspase 3 / β-tubulin (% control)
IL-1β (ng/mL):
0
10
D
0ng/mL IL-1β + 5.5mmol/L glucose
10ng/mL IL-1β + 25mmol/L glucose
Ad-GFP
Ad-SENP1
Ad-GFP
Ad-SENP1
CC3
merge
CC3+, GFP+ (% total GFP+)
IL-1β + HG: - + - + - +
donor 1
donor 2
donor 3

Figure 19. SENP1 sensitizes mouse islets to IL-1β and high glucose-induced impairments in $[Ca^{2+}]_i$ handling. ►

Mouse islets were transduced with Ad-GFP or Ad-SENP1 cultured in 11.1 or 25 mmol/l glucose and treated with vehicle or IL-1β (10 ng/ml) for 24 hours. **A:** Representative responses of $[Ca^{2+}]_i$ in mouse islets stimulated with glucose (11 mmol/l; arrow). **B:** Baseline $[Ca^{2+}]_i$ measurements during low glucose (0.5 mmol/l) stimulation (*n*=8, 9, 6, 5; 5 experiments). **C:** Fold increase in glucose-stimulated $[Ca^{2+}]_i$ (GSCa) responses (*n*=9, 9, 6, 6; 5 experiments). **D:** Change in area under the curve (ΔAUC) of $[Ca^{2+}]_i$ responses (9, 9, 6, 5; 5 experiments). *n* values correspond to data points from left to right, respectively. *P<0.05 as indicated.

Figure 19.


fura-red, AM ratio
Ad-GFP
vehicle
Ad-SENP1
vehicle
Ad-GFP
IL-1β + 25G
Ad-SENP1
IL-1β + 25G
400s
baseline
Ad-GFP
Ad-SENP1
IL-1β:
glucose:
11.1
25
GSCa
ΔAUC (fura-red ratio x time)
*

3.4.c SENP1 increases ER stress and enhances IL-1β-induced expression of NFκB target gene *Nos2*.

To further elucidate the mechanism of SENP1-induced apoptosis, expression of genes involved in ER stress were assessed (Eizirik et al., 2013). When insulin demand exceeds ER capacity, protein folding by the ER becomes impaired and the cytoprotective unfolded protein response (UPR) is initiated (Walter and Ron, 2011). The UPR will attempt to alleviate ER stress by reducing protein translation, increasing transcription of chaperone proteins, and inducing degradation of misfolded proteins. Under non-stimulatory conditions, association of upstream ER stress activators to binding immunoglobulin protein (BiP) inhibit activation of UPR. However, BiP preferentially binds to misfolded proteins and when ER capacity is overwhelmed and misfolded proteins accumulate, BiP dissociates from ER stress activators, allowing their subsequent activation. Induction of the protein kinase RNA-like ER kinase (PERK) branch of the ER stress pathway up-regulates activating transcription factor 4 (ATF4) and the pro-apoptotic C/EBP homologous protein (CHOP) (Allagnat et al., 2012). Overexpression of SENP1 induced ER stress, as evidenced by increased *Chop* (n=10; Figure 20A) and *Atf4* (n=10; Figure 20B) expression. There was a trend for SENP1 to increase the secreted proinsulin-to-insulin ratio from mouse islets (n=7; Figure 20C) suggesting that SENP1 may also induce ER stress in primary cells.

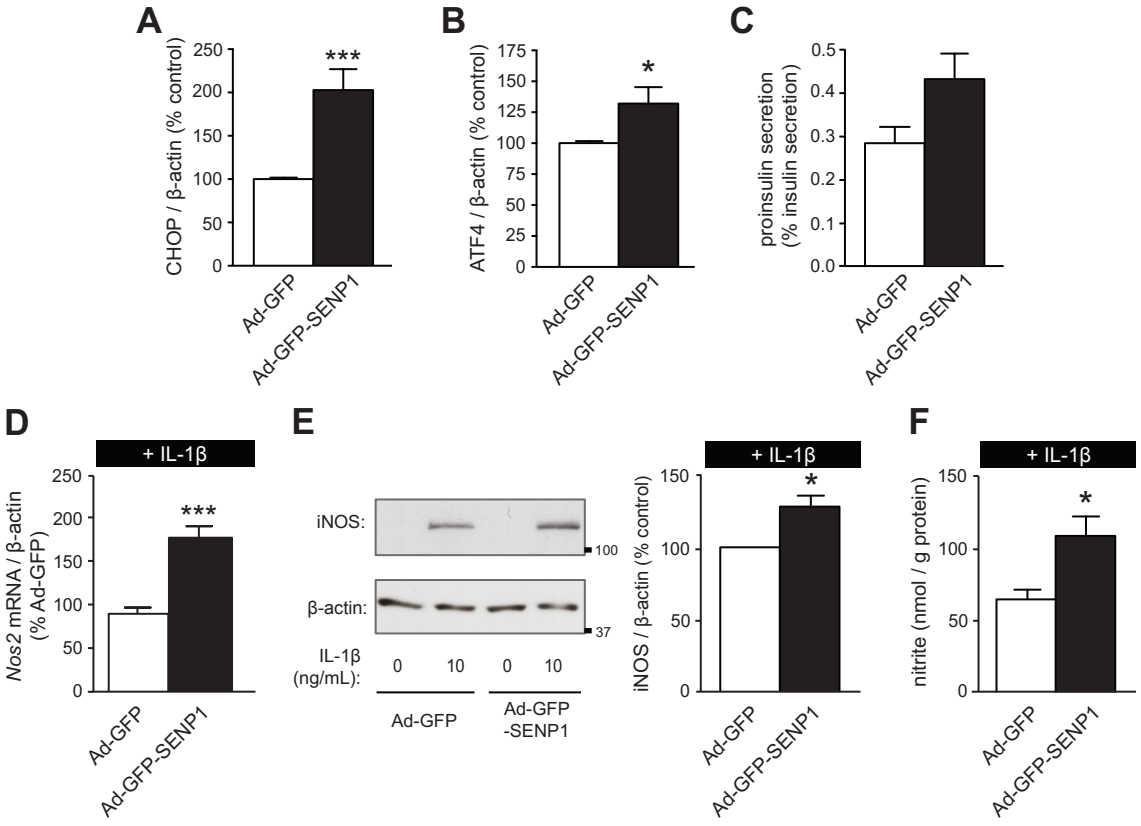
SENP1 was also able to enhance cell death in the presence of IL-1β—a well-established inducer of pancreatic β-cell death and dysfunction (Mandrup-Poulsen et al., 1986; Rabinovitch et al., 1990). The major pathway of IL-1β-induced apoptosis is through NFκB, where IL-1β-induced nitric oxide production, insulin secretory dysfunction, and apoptosis are inhibited when NFκB activity is reduced (Giannoukakis et al., 2000). To examine whether

SENP1 was able to enhance IL-1β-induced activation of this pathway, expression of the NFκB target gene *Nos2* (Darville and Eizirik, 1998; Heimberg et al., 2001) was measured. Overexpression of SENP1 enhanced IL-1β-induced mRNA expression of *Nos2* (*n*=4; Figure 20D), iNOS protein expression (*n*=3; Figure 20E), and nitrite production (*n*=3; Figure 20F) in INS 832/13 cells.

Figure 20. SENP1 induces ER stress and enhances IL-1β-induced transcription of NFκB target genes. ►

A, B: INS 832/13 cells were transduced with Ad-GFP or Ad-GFP-SENP1. RT-PCR detection and quantification of *Chop* **(A)** or *Atf4* **(B)** mRNA expression, normalized to β-actin mRNA, was determined. Levels of mRNA expression as a percentage of Ad-GFP control cells are shown; data collection and analysis by MF. **C:** Proinsulin secretion as a percentage of insulin secretion at 2.8 mmol/l glucose from mouse islets transduced with Ad-GFP or Ad-GFP-SENP1; data collection by AFS. **D:** INS 832/13 cells transduced with Ad-GFP or Ad-GFP-SENP1 were treated with IL-1β (10 ng/ml) for 6 h. RT-PCR detection and quantification of *Nos2* mRNA expression, normalized to β-actin mRNA, was determined. Levels of *Nos2* as a percentage of IL-1β-treated control cells are shown; data collection and analysis by MF. **E:** Immunoblotting for iNOS and β-actin (left) from INS 832/13 cells transduced with Ad-GFP or Ad-GFP-SENP1 and treated with IL-1β for 24 h. Quantification by densitometry relative to IL-1β-treated control cells is shown (right). **F:** Nitrite accumulation in culture media normalized to protein content of INS 832/13 cells transduced with Ad-GFP or Ad-GFP-SENP1 and treated with IL-1β (10 ng/ml) for 24 h. *P<0.05, ***P< 0.005.

Figure 20.


A
CHOP / β-actin (% control)
250
200
150
100
50
0
***
Ad-GFP
Ad-GFP-SENP1
B
ATF4 / β-actin (% control)
175
150
125
100
75
50
25
0
*
Ad-GFP
Ad-GFP-SENP1
C
proinsulin secretion
(% insulin secretion)
0.5
0.4
0.3
0.2
0.1
0.0
Ad-GFP
Ad-GFP-SENP1
D
+ IL-1β
Nos2 mRNA / β-actin
(% Ad-GFP)
250
200
150
100
50
0
***
Ad-GFP
Ad-GFP-SENP1
E
iNOS:
β-actin:
100
37
IL-1β
(ng/mL):
0
10
0
10
Ad-GFP
Ad-GFP
-SENP1
+ IL-1β
iNOS / β-actin (% control)
150
100
50
0
*
Ad-GFP
Ad-GFP-SENP1
F
+ IL-1β
nitrite (nmol / g protein)
150
100
50
0
*
Ad-GFP
Ad-GFP-SENP1

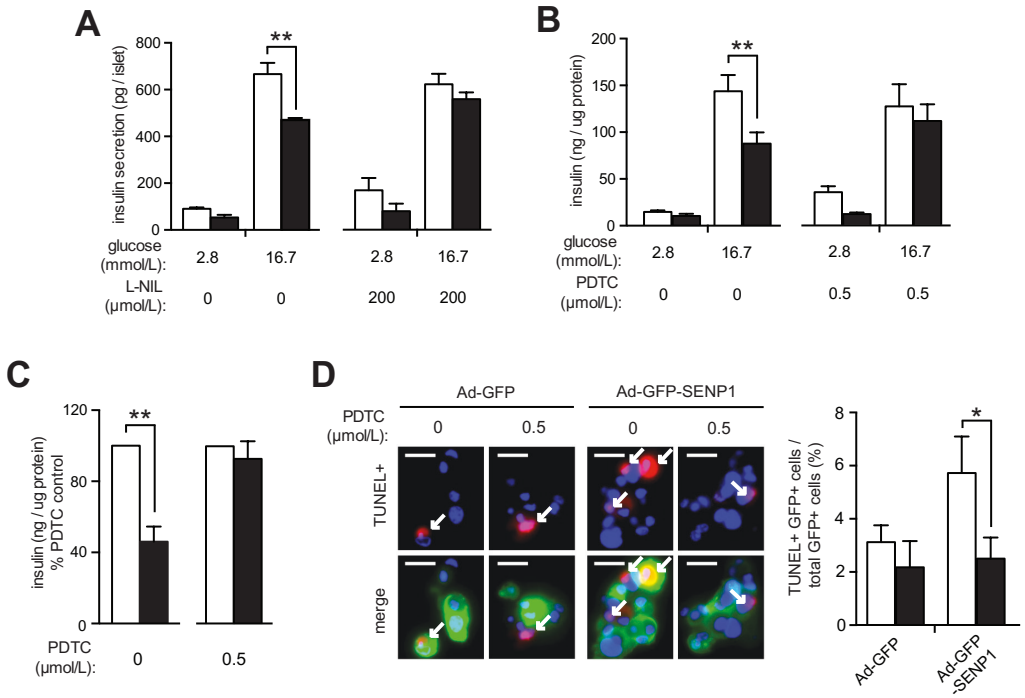
3.4.d Pharmacological inhibition of iNOS or NFκB ameliorates SENP1-induced secretory dysfunction in human islets.

To examine whether increased iNOS or NFκB mediates the impaired secretory response associated with up-regulation of SENP1, intact mouse islets were concomitantly transduced with Ad-GFP or Ad-GFP-SENP1 and treated with either the iNOS inhibitor, L-NIL (Tanioka et al., 2011), or the NFκB inhibitor, PDTC (Flodström et al., 1996). SENP1-induced secretory dysfunction was no longer observed following treatment of mouse islets with either L-NIL (*n*=4; Figure 21A) or NFκB (*n*=6; Figure 21B). Although PDTC appeared to prevent the inhibitory effects of SENP1 on insulin secretion, it is important to note that no significant difference in insulin released from Ad-SENP1-treated islets in either the absence or presence of PDTC was observed. PDTC protected against SENP1-induced dysfunction in intact human islets (*n*=3; Figure 21C), where the cytoprotection afforded by PDTC was associated with a reduction in human islet cell death when SENP1 was upregulated (*n* = 4; Figure 21D).

Figure 21. Inhibition of NFκB reduces SENP1-induced secretory dysfunction in islets. ►

A: Insulin secretion per islet from mouse islets transduced with Ad-GFP (open bars) or Ad-GFP-SENP1 (filled bars) and concomitantly treated with L-NIL; data collection by AFS. **B:** Insulin secretion normalized to protein content from mouse islets transduced with Ad-GFP (open bars) or Ad-GFP-SENP1 (filled bars) and concomitantly treated with PDTC; data collection by AFS. **C:** Insulin secretion at 16.7 mmol/l glucose normalized to protein content from intact human islets transduced with Ad-GFP (open bars) or Ad-GFP-SENP1 (filled bars) and concomitantly treated with PDTC as a percentage of PDTC control. **D:** Dispersed human islet cells transduced with Ad-GFP or Ad-GFP-SENP1 and concomitantly treated with (filled bars) or without (open bars) PDTC and stained for TUNEL (red), nuclei (DAPI, blue), and GFP (green). Representative images (left) and quantification of GFP+ and TUNEL+ cells (arrows), as a percentage of total GFP+ cells (right), are shown. Merged images represent merge of TUNEL, DAPI, and GFP immunofluorescence; note that GFP immunofluorescence alone is not shown. Scale bars represent 20 microns. **P< 0.01.

Figure 21.


A
insulin secretion (pg / islet)
800
600
400
200
0
**
glucose (mmol/L):
2.8
16.7
2.8
16.7
L-NIL (µmol/L):
0
0
200
200
B
insulin (ng / ug protein)
200
150
100
50
0
**
glucose (mmol/L):
2.8
16.7
2.8
16.7
PDTC (µmol/L):
0
0
0.5
0.5
C
insulin (ng / ug protein) % PDTC control
120
80
40
0
**
PDTC (µmol/L):
0
0.5
D
Ad-GFP
Ad-GFP-SENP1
PDTC (µmol/L):
0
0.5
0
0.5
TUNEL+
merge
TUNEL+ GFP+ cells / total GFP+ cells (%)
8
6
4
2
0
*
Ad-GFP
Ad-GFP -SENP1

3.4.e Enhanced SUMOylation protects against stimulus-induced cell death and reduces *Nos2* expression and nuclear translocation of NFκB.

Given that overexpressed SENP1 promoted islet cell death, we knocked down this deSUMOylating enzyme using siSENP1 duplexes (Figure 22A). Knockdown of SENP1 reduced IL-1β-induced *Nos2* mRNA expression (*n*=3; Figure 22B) and apoptosis (*n*=5; Figure 22C) in INS 832/13 cells treated with IL-1β for 6 or 24 hours, respectively. DeSUMOylation mediated by SENP1 has the greatest specificity for SUMO1 conjugates (Kolli et al., 2010; Sharma et al., 2013); therefore, we up-regulated SUMO1 using Ad-GFP-SUMO1 (Figure 23A) or Ad-SUMO1 [previously characterized (Dai et al., 2011)]. In INS 832/13 cells, Ad-GFP-SUMO1 protected against IL-1β-induced cell death (*n*=5 experiments (2937 cells); Figure 23B). To determine if this was due to decreased apoptosis, cleaved caspase 3 levels were measured in human islets and INS 832/13 cells by immunohistochemistry or immunoblotting, respectively. Infected cells were identified by the presence of GFP. Ad-SUMO1 reduced basal (*n*=4) and IL-1β-induced apoptosis (*n*=4) in INS 832/13 cells (Figure 23C). The SUMO1-mediated reduction in apoptosis was associated with a reduction in stimulus-induced nuclear translocation of NFκB subunit p65 (*n*=3; Figure 23D). Ad-SUMO1 also reduced stimulus-induced caspase 3 cleavage in all human donors examined (*n*=3 donors (1027 cells); Figure 23E).

Figure 22. Knockdown of SENP1 protects against IL-1β-induced apoptosis. ►

A: Knockdown of endogenous SENP1 and the loading control β-actin in INS 832/13 cells. **B:** INS 832/13 cells transfected with siScram or siSENP1 were treated with IL-1β (10 ng/ml) for 6 h. RT-PCR detection and quantification of *Nos2* mRNA expression normalized to β-actin mRNA was determined. Levels of *Nos2* as a percentage of IL-1β-treated control cells are shown; data collection and analysis by MF. **C:** Immunoblotting for cleaved caspase 3 and β-tubulin (left) from INS 832/13 cells transfected with siScram or siSENP1 and treated with IL-1β for 24 h. Quantification by densitometry relative to IL-1β-treated control cells is shown (right). **P<0.01, ***P<0.005.

Figure 22.

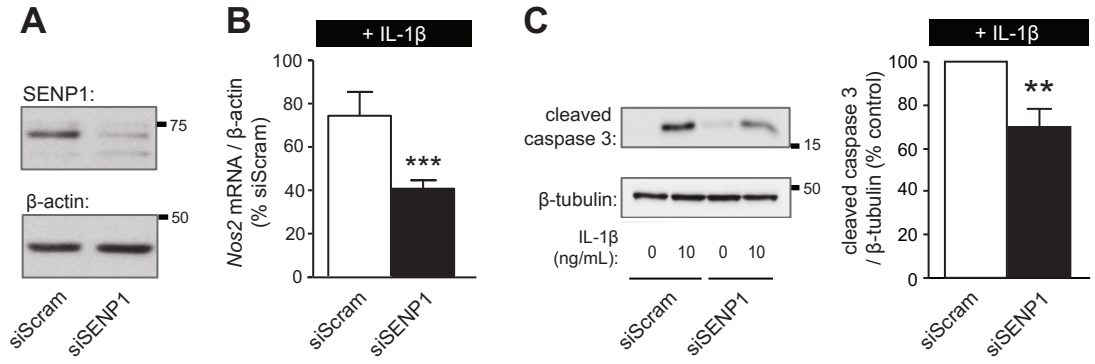
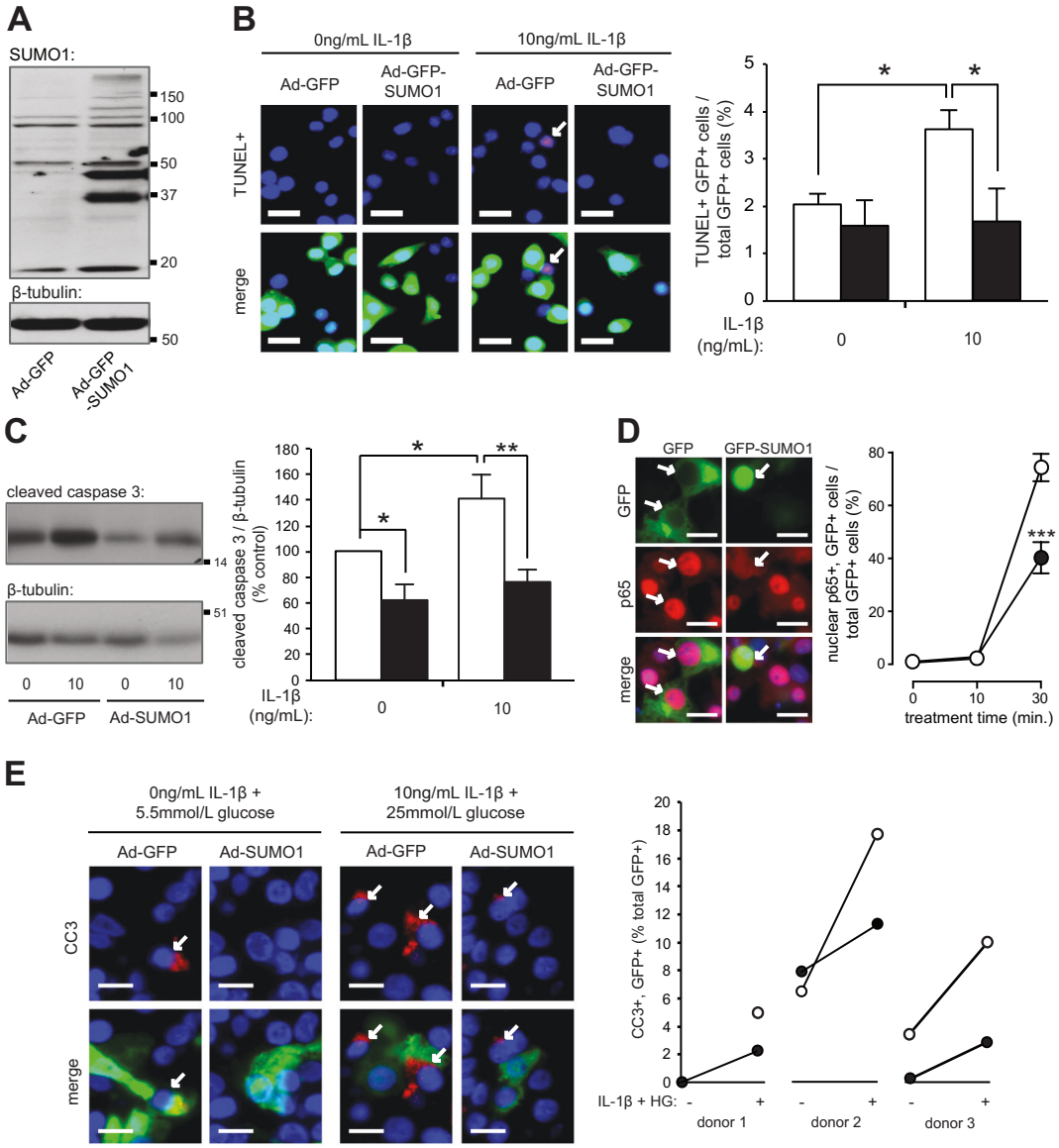

A
SENP1:
75
β-actin:
50
siScram
siSENP1
B
+ IL-1β
Nos2 mRNA / β-actin
(% siScram)
100
80
60
40
20
0
***
siScram
siSENP1
C
cleaved
caspase 3:
15
β-tubulin:
50
IL-1β
(ng/mL):
0 10 0 10
siScram
siSENP1
+ IL-1β
cleaved caspase 3
/ β-tubulin (% control)
100
80
60
40
20
0
**
siScram
siSENP1

Figure 23. SUMO1 protects against stimulus-induced apoptosis. ►

A: Endogenous and over-expressed SUMO1 and the loading control β-tubulin from INS 832/13 cells. **B:** INS 832/13 cells transduced with Ad-GFP (open bars) or Ad-GFP-SUMO1 (filled bars) treated with IL-1β for 24 hours and stained for TUNEL (red), nuclei (DAPI, blue), and GFP (green). Representative images (left) and quantification of GFP+ and TUNEL+ cells (arrows), as a percentage of total GFP+ cells (right), are shown. Merged images represent merge of TUNEL, DAPI, and GFP immunofluorescence; note that GFP immunofluorescence alone is not shown. Scale bars represent 15 microns. **C:** Immunoblotting for cleaved caspase 3 (CC3) and β-tubulin (left) from INS 832/13 cells transduced with Ad-GFP (open bars) or Ad-GFP-SUMO1 (filled bars) and treated with IL-1β for 24 hours. Quantification by densitometry relative to Ad-GFP group (right). **D:** INS 832/13 cells transfected with GFP (open circles) or GFP-SUMO1 (filled circles) and treated with high glucose (25 mmol/l) and IL-1β (10 ng/ml), as indicated. Cells were immunostained for p65 (red) and nuclei (DAPI; blue). Representative images following 30-min stimulation (left) and quantification of GFP+ cells (arrows) with nuclear p65 as a percentage of GFP+ cells (right) are shown. Scale bars represent 15 microns; image acquisition by GP. **E:** Human islets transduced with Ad-GFP (open circles) or Ad-SUMO1 (filled circles) were treated, as indicated, for 48 hours. Islets sections were immunostained for GFP (green), CC3 (red), and nuclei (DAPI; blue). Representative images (left) and GFP+ and CC3+ cells (arrows), as a percentage total GFP+ cells (right), are shown. Merged images represent merge of CC3, DAPI, and GFP immunofluorescence; note that GFP immunofluorescence alone is not shown. Scale bars represent 10 microns. *P<0.05, **P<0.01, ***P<0.005.

Figure 23.


A
SUMO1:
150
100
50
37
20
β-tubulin:
50
Ad-GFP
Ad-GFP -SUMO1
B
0ng/mL IL-1β
10ng/mL IL-1β
Ad-GFP
Ad-GFP-SUMO1
Ad-GFP
Ad-GFP-SUMO1
TUNEL+
merge
TUNEL+ GFP+ cells / total GFP+ cells (%)
IL-1β (ng/mL):
0
10
C
cleaved caspase 3:
14
β-tubulin:
51
0
10
0
10
Ad-GFP
Ad-SUMO1
cleaved caspase 3 / β-tubulin (% control)
IL-1β (ng/mL):
D
GFP
GFP-SUMO1
GFP
p65
merge
nuclear p65+, GFP+ cells / total GFP+ cells (%)
treatment time (min.)
E
0ng/mL IL-1β + 5.5mmol/L glucose
10ng/mL IL-1β + 25mmol/L glucose
Ad-GFP
Ad-SUMO1
Ad-GFP
Ad-SUMO1
CC3
merge
CC3+, GFP+ (% total GFP+)
IL-1β + HG:
donor 1
donor 2
donor 3

3.5 DISCUSSION

Reduction in β-cell mass characteristic of diabetic patients is thought to arise, at least in part, following increased β-cell apoptosis (Butler et al., 2003). Regulators of apoptotic pathways, particularly those capable of protecting against stimulus-induced cell death, represent potential targets for the preservation of β-cell mass. Here, we identify the SUMOylation pathway as a novel regulator of apoptosis in pancreatic β-cells.

SUMO1 conjugates to several β-cell proteins (Dai et al., 2011; Manning Fox et al., 2012; Vergari et al., 2012). The effects of SUMO1 on β-cell function have primarily been examined in the context of insulin secretion. Whereas potential insulinotropic effects of SUMO1 on K^+ channels and glucokinase have been identified (Aukrust et al., 2013; Dai et al., 2009), a majority of the known SUMO1 modifications promote secretory dysfunction (Dai et al., 2011; Kishi et al., 2003; Rajan et al., 2012; Shao and Cobb, 2009). Overall, global SUMO1 up-regulation reduces insulin secretion from both rodent and human islets (Dai et al., 2011; Rajan et al., 2012). In contrast, the present study examines the role of SUMO1 on β-cell viability and establishes SUMO1 as an anti-apoptotic protein conferring protection against IL-1β-induced β-cell death. This is consistent with its cytoprotective role against heat shock- (Kim et al., 2011), cytokine- (Okura et al., 1996), and Fas-ligand- (Meinecke et al., 2007; Okura et al., 1996) induced cell death in other cell types and is in contrast to its detrimental effects on cell viability in others (Feligioni et al., 2011). Although it remains unknown whether SUMOylation is protective against other challenges, such as stronger inflammatory environment conferred by commonly used cytokine cocktails, the present study nonetheless establishes a positive role for SUMOylation in pancreatic β-cells.

We have also identified SUMOylation as a negative regulator of IL-1β-induced iNOS expression in INS 832/13 cells. Although the role of iNOS in β-cell death remains

controversial (Bedoya et al., 2012; Eizirik et al., 1996b), several studies have demonstrated that reduced iNOS expression or activity decreases cytokine-mediated cytotoxicity (Arnush et al., 1998; Corbett et al., 1993; Liu et al., 2000). Whether the protective effect of SUMOylation depends on reduced iNOS expression, however, has yet to be determined. In β-cells, cytokine-induced iNOS transcription and subsequent nitric oxide production is regulated through the nuclear transcription factor, NFκB (Flodström et al., 1996; Giannoukakis et al., 2000; Heimberg et al., 2001). NFκB is a key mediator of cytokine-induced β-cell cytotoxicity (Giannoukakis et al., 2000). When NFκB activity is reduced in human islets, stimulus-induced nitric oxide production, insulin secretory dysfunction, and apoptosis are inhibited (Giannoukakis et al., 2000). Post-translational SUMOylation is known to inhibit NFκB activity in other cell types (Desterro et al., 1998; Liu et al., 2009). That SUMOylation reduces stimulus-induced nuclear translocation of the NFκB target gene *Nos2* suggests that enhanced SUMOylation induces cytoprotection by reducing activation of NFκB.

SENP1 impairs insulin secretion from pancreatic islets in apparent contradiction with the inhibitory effects of enhanced SUMOylation (Dai et al., 2011; Kishi et al., 2003; Rajan et al., 2012; Shao and Cobb, 2009). It should be noted that the mechanism(s) by which over-expressed SUMO1 and SENP1 inhibit secretory function are quite distinct. Increased SUMO1 regulates secretory machinery directly by inhibiting GLP-1 receptor signaling and exocytosis (Dai et al., 2011; Rajan et al., 2012), while at the same time protecting β-cells from apoptosis. Upregulation of SENP1, on the other hand, induces ER stress and enhances IL-1β-induced iNOS expression and activity while promoting dysfunction through induction of apoptosis. Even though SENP1 can acutely promote β-cell exocytosis (Dai et al., 2011)

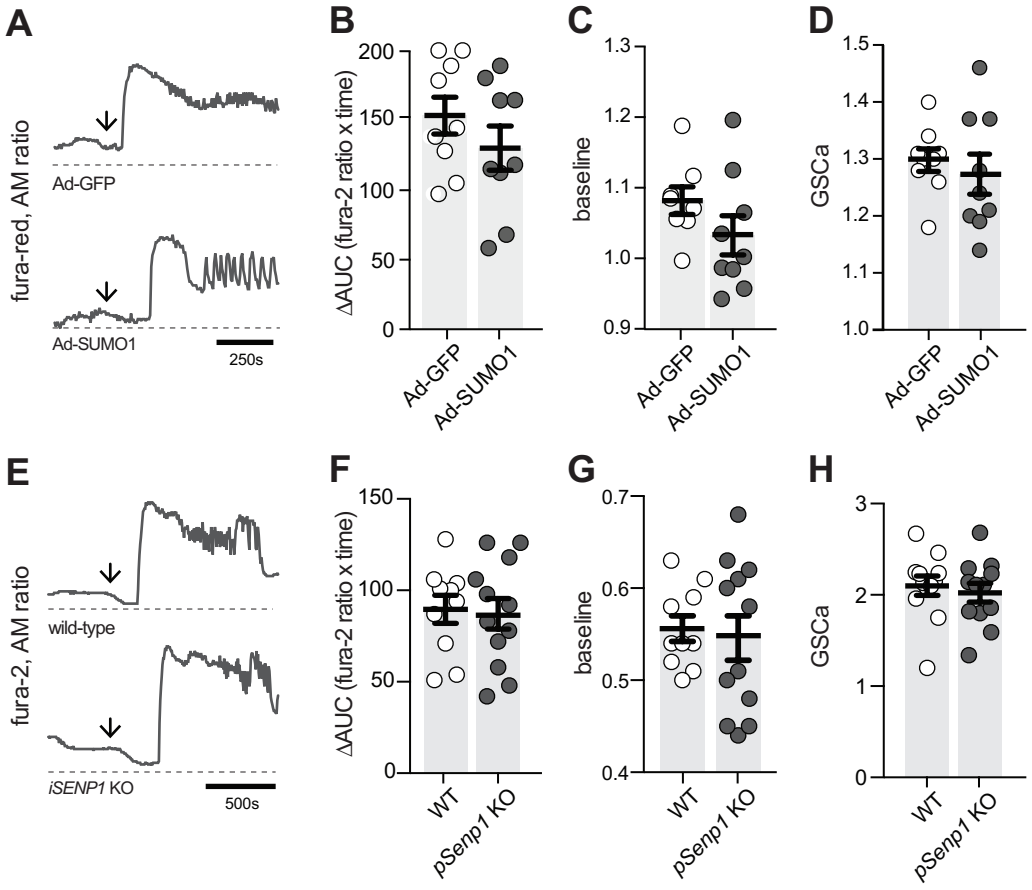
and plays a role in metabolic sensing (Ferdaoussi et al., 2015; Vergari et al., 2012), the deleterious effect on islet viability overwhelms these effects when SENP1 is overproduced.

In the present study, SENP1-induced secretory dysfunction was not observed in the presence of PDTC, an inhibitor of NFκB (Eizirik et al., 1996b; Melloul, 2008). While this suggests that SENP1-mediated dysfunction is due to reduced viability, that PDTC itself has effects on secretion may be confounding these results. Nevertheless, the present findings are in agreement with several others demonstrating an association between reduced viability and impaired insulin secretion (Emamaullee et al., 2008; Hui et al., 2005; Nakano et al., 2004; Rivas-Carrillo et al., 2007; Song et al., 2013). Additionally, the protective effect of PDTC on insulin secretion occurs in parallel to a reduction in human islet cell death. We also find that the $[Ca^{2+}]_i$ response is impaired following SENP1 upregulation, consistent with the known effects of reduced viability on $[Ca^{2+}]_i$ handling (Carter et al., 2009; Dula et al., 2010; Jahanshahi et al., 2009). That the effects of SENP1 on $[Ca^{2+}]_i$ are likely secondary to reduced viability are further supported by observations that over-expression of SUMO1 (Figure 24A) or knockout of *Senp1* (Figure 24B) has no effect on $[Ca^{2+}]_i$. Taken together, these data suggest the SENP1-induced secretory dysfunction is secondary to reduced islet viability and impaired $[Ca^{2+}]_i$ handling rather than a direct effect on the secretory machinery, as is observed following upregulation of SUMO1.

Figure 24. $[Ca^{2+}]_i$ is unaffected by enhanced SUMOylation. ►

A-D: Mouse islets transduced with Ad-GFP or Ad-SUMO1 treated with vehicle or IL-1β (10 ng/ml) for 24 hours. **(A)** Representative responses in $[Ca^{2+}]_i$ in mouse islets stimulated with glucose (11 mmol/l; arrow). **(B)** Change in area under the curve (ΔAUC) of $[Ca^{2+}]_i$ responses (9, 9; 5 experiments). **(C)** Baseline $[Ca^{2+}]_i$ measurements during low glucose (0.5 mmol/l) stimulation (*n*=8, 9; 5 experiments). **(D)** Fold increase in glucose-stimulated $[Ca^{2+}]_i$ (GSCa) responses (*n*=9, 9; 5 experiments). **E-H:** Islets were isolated from pancreatic-specific knockout of *Senp1* (*pSenp1* KO) or from their wild-type (WT) littermates. $[Ca^{2+}]_i$ was assessed using methods described in Section 2.3a and 2.3c, unless otherwise indicated. **(E)** Representative responses in $[Ca^{2+}]_i$ in mouse islets stimulated with glucose (11 mmol/l; arrow). **(F)** Change in area under the curve (ΔAUC) of $[Ca^{2+}]_i$ responses (12, 12; 4 experiments). **(G)** Baseline $[Ca^{2+}]_i$ measurements during low glucose (0.5 mmol/l) stimulation (*n*=10, 12; 4 experiments). **(H)** Fold increase in glucose-stimulated $[Ca^{2+}]_i$ (GSCa) responses (*n*=10, 12; 4 experiments). *n* values correspond to data points from left to right, respectively.

Figure 24.


A
fura-red, AM ratio
Ad-GFP
Ad-SUMO1
250s
B
ΔAUC (fura-2 ratio x time)
200
150
100
50
0
Ad-GFP
Ad-SUMO1
C
baseline
1.3
1.2
1.1
1.0
0.9
Ad-GFP
Ad-SUMO1
D
GSCa
1.5
1.4
1.3
1.2
1.1
1.0
Ad-GFP
Ad-SUMO1
E
fura-2, AM ratio
wild-type
iSENP1 KO
500s
F
ΔAUC (fura-2 ratio x time)
150
100
50
0
WT
pSenp1 KO
G
baseline
0.7
0.6
0.5
0.4
WT
pSenp1 KO
H
GSCa
3
2
1
0
WT
pSenp1 KO

3.6 STUDY LIMITATIONS

In the present study, SUMOylation was enhanced by the adenoviral-mediated up-regulation of SUMO1 resulting in increased SUMOylation of numerous target proteins (Figure 23A). Although deSUMOylation by SENP1 was not quantified in the present study, that SENP1 inhibits the effects of co-infused SUMO1 on β-cell exocytosis (Dai et al., 2011) and reduces SUMO1 conjugation of target proteins including Kv2.1 (Dai et al., 2009) and synaptotagmin VII (Dai et al., 2011) demonstrates the deSUMOylatory capability of SENP1.

Given the large number of SUMOylated targets, the consequences of SUMO1 overexpression on β-cell survival are likely secondary to activation or deactivation of several cellular pathways. Whether SUMO1 directly regulates IL-1 signaling or whether it antagonizes the effects of IL-1β by modulating IL-1β-independent pathways cannot be conclusively determined in the present study. SUMOylation mutants, engineered by a substitution of the consensus sequence lysine residues (Desterro et al., 1998), will be required to identify the direct targets of SUMO1 conjugation that mediate the effects of IL-1 signaling on β-cell death. However, that SUMOylation reduces IL-1β-induced activation of NFκB and *Nos2* expression suggests that the effects are, at least in part, due to an inhibition of the IL-1β ⟶ NFκB pathway.

While SUMOylation reduces IL-1β and high glucose-induced apoptosis in *human* islet cells, the current study could not examine the effects of SUMO1 conjugation on apoptosis induced by IL-1β alone. Consistent with previous reports (Eizirik et al., 1993), cytotoxicity in human islets induced by IL-1β alone was undetectable. Additional stimuli, in this case high glucose, was required to observe a consistent induction of cell death, thereby confounding the ability to examine the regulatory role of SUMO1 on IL-1 signaling.

3.7 CONCLUDING REMARKS

The current study identifies SUMOylation as a regulator of IL-1 signaling in insulin-secreting cells and proposes that this occurs secondary to modulation of NFκB activity. However, whether SUMO1 regulates the acute insulinotropic effects of this cytokine, and the consequences of this regulation in vivo, have yet to be determined. To fully understand these processes, interactions between SUMO1 and IL-1 signaling, as well as the spatial and temporal mechanisms regulating SUMOylation itself, will require elucidation.

It is evident from numerous scientific studies that inhibition of chronic IL-1 signaling improves β-cell function and viability in vitro. Results of the current chapter support these findings, and demonstrate that IL-1β-induced stress is reduced by the over-expression of SUMO1. While the efficacy of systemic IL-1 inhibition in vivo requires further exploration, results of this study suggest that targeted inhibition of chronic IL-1 signaling by SUMO1 in vitro may effectively reduce inflammation-induced β-cell death. The following chapter examines the therapeutic potential of SUMO1 in the preservation of functional islet mass during the inflammatory-associated process of islet isolation.

CHAPTER 4.

Enhancing SUMOylation to reduce pre-transplantation-associated stress.

Co-author contributions to the figures presented are acknowledged in the figure legends by co-author initials.

4.1 ABSTRACT

Pre-transplant islet loss is a limiting factor in the therapeutic feasibility of islet transplantation. Islet isolation activates pro-apoptotic inflammatory and hypoxia response pathways resulting in significant loss of islet cell mass. In the preceding chapter, and consistent with its role as a negative regulator of inflammation and cell death in other cell types, SUMO1 was identified as an anti-apoptotic protein capable of reducing stimulus-induced apoptosis in human islet cells. While this suggests a potential role for SUMO1 in the preservation of pre-transplant islet mass, the cytoprotective effects of SUMOylation against isolation-induced stress have yet to be determined. In the present study, a membrane permeable, biologically-active SUMO1 peptide was produced by the fusion of SUMO1 to a TAT_{48-57} transduction domain. SUMOylation induced by TAT-SUMO1 reduced markers of inflammatory signaling, ER stress, and hypoxia in an insulin-secreting cell line treated with IL-1β. Preliminary findings that TAT-SUMO1 reduces isolation-induced up-regulation of NFκB and HIF1α target genes in freshly isolated human islet cells support a cytoprotective role of SUMO1 against isolation-induced stress.

4.2 INTRODUCTION

T1D results from the autoimmune-mediated destruction of pancreatic β-cells (Achenbach et al., 2005). Due to the loss of β-cell glucoregulatory mechanisms, numerous T1D patients continue to experience detrimental episodes of hypo- and hyperglycemia despite the use of exogenous insulin therapy (Bertuzzi et al., 2007). Glucose-responsive insulin secretion can be restored in these patients by the reintroduction of β-cells through islet transplantation. Islet transplantation has proven successful in inducing short-term insulin-independence, and more importantly, in reducing erratic blood glucose fluctuations associated with T1D (Shapiro et al., 2000). Indeed, HbA_{1c} levels and hypoglycemic episodes are markedly reduced post-transplantation (Alejandro et al., 2008; Barton et al., 2012; Ryan et al., 2005) and in comparison to conventional insulin therapy (Vantyghem et al., 2009; Warnock et al., 2008).

Islet transplantation success depends on the infusion of a threshold islet mass (Weir et al., 1990). In humans, a minimum of 9,000 islet equivalents per kg of body weight (Ryan et al., 2001), or infusion of 630,000 islet equivalents per 70 kg individual, is required to achieve insulin independence. However, current isolation procedures seldom result in isolation of more than 600,000 islet equivalents per pancreas (Lyon et al., 2016; Shapiro et al., 2000) necessitating the transplant of multiple donor organs to achieve threshold mass. Given the shortage of suitable donor tissue, multi-donor transplantation requirements limit the widespread use of islet transplant therapy.

Pre-transplantation islet loss represents a significant barrier to single-donor transplantation, with islet cell death occurring during organ collection, cold ischemia of organ transport, and through the islet isolation procedure (Kanak et al., 2014). Islet isolation alone reduces human islet mass by 14% within 60-hours of isolation (Bottino et al., 2004),

with apoptotic markers present in over 30% of the remaining islet cells (Paraskevas et al., 2000). During isolation, islets are dissociated from the exocrine pancreas through enzymatic digestion and mechanical agitation of pancreatic tissue (McCall and Shapiro, 2014). Isolation-induced losses in islet mass are attributed to activation of pro-apoptotic NFκB (Abdelli et al., 2004; Bottino et al., 2004) and JNK (Abdelli et al., 2004) secondary to mechanical and chemical stress and to the induction of devascularization-induced hypoxia (Kanak et al., 2014). Consequently, strategies inhibiting pro-apoptotic signaling may reduce requirements of multi-donor transplantation by preserving functional islet mass.

SUMO1 is an anti-apoptotic protein capable of protecting human islet cells against IL-1β and high glucose-induced apoptosis (Figure 23). While the cytoprotective actions of SUMO1 are attributed to a reduction in NFκB activity (Figure 22, Figure 23), SUMO1 reduces heat shock-induced activation of pro-apoptotic JNK (Kim et al., 2011) and dampens transcription of inflammatory response genes (Pascual et al., 2005) in other cell types. Furthermore, SUMOylation reduces hypoxia-inducible factor 1α (HIF1α)-regulated gene transcription (Cheng et al., 2007), with supra-physiological activation of HIF1α associated with β-cell toxicity (Cheng et al., 2010).

SUMO1, however, is unable to permeate cell membranes rendering it relatively ineffective as a molecular therapy in its physiological form. Peptide transduction domains have been used to overcome the therapeutic limitation of cell impermeability. One such domain, the 10-amino acid HIV-transactivator of transcription (TAT_{48-57}) sequence, is capable of efficiently transducing biological cargo across cell membranes (Vivès et al., 1997). TAT_{48-57} peptides are capable of transducing proteins into a variety of mammalian tissues (Schwarze et al., 1999), across monolayer culture (Bonny et al., 2001), and into isolated pancreatic islets (Abdelli et al., 2007).

The present study examined the cytoprotective potential of TAT-SUMO1 against isolation-induced β-cell stress. SUMO1 fusion to the TAT_{48-57} transduction domain imparted cell permeability to SUMO1 and did not impair its biological activity. Enhanced SUMOylation induced by TAT-SUMO1 reduced IL-1β-induced NFκB, ER stress, and hypoxia-associated gene transcription in an insulin secreting cell line. Preliminary results indicate that TAT-SUMO1 reduces isolation-induced inflammatory and hypoxia-associated signaling in human islets cells. Together, these data confirm the anti-apoptotic actions of SUMO1 and support further investigation of TAT-SUMO1 as a small-peptide inhibitor of isolation-induced islet loss.

4.3 MATERIALS AND METHODS

4.3.a Cells and cell culture.

INS 832/13 cells, a rat insulinoma cell line from C. Newgard (Duke University, Durham, North Carolina, USA), were cultured in RPMI 1640 containing 11.1 mmol/l glucose with 10% FBS, 10 mmol/l HEPES, 0.29 mg/ml L-glutamine, 1 mmol/l sodium pyruvate, 50 μmol/l β-mercaptoethanol, and 100 U/ml of penicillin/streptomycin. Human islets were isolated as previously described (Lyon et al., 2016) from non-diabetic donor pancreata at the Alberta Diabetes Institute IsletCore (http://www.bcell.org/IsletCore.html) at the University of Alberta (Edmonton, Alberta, Canada) and were cultured in DMEM (5.5 mmol/l) with L-glutamine, 100 mg/ml sodium pyruvate, 10% FBS, and 100 units/ml penicillin/streptomycin. In total, islets from 4 human donors were examined in this study (age: 60.5 ± 3.28; BMI: 26.3 ± 1.97; HbA_{1c}: 5.8 ± 0.18). For single cell experiments, human islets were dispersed by shaking in cell dissociation buffer (Gibco, Thermo Scientific) and plated in 35-mm culture dishes. Intact islets, INS 832/13 cells, or dispersed human cells were cultured at 37°C and 5% CO_2.

4.3.b Peptide generation.

Cell penetrating TAT peptide, derived from TAT_{48-57} of HIV, was fused to the N-terminus of SUMO1 or mCherry. The recombinant proteins containing TAT peptide were prepared by E.coli expression system using Rosetta (DE3)pLysS strain (Novagen/EMD Millipore). SUMO1 cDNA containing a stop codon was excised from the pIRES-EGFP-C1 vector (a gift from Dr. Steven Ogg, University of Alberta). The expression vector of TAT-SUMO1 was prepared by inserting cDNA into a pET28.2 TAT-Cre plasmid (Addgene). PCR

was used to prepare mCherry cDNA using Cherry-LacRep plasmid (Addgene) as a template. The PCR fragment of mCherry was inserted into the pET28.2 TAT-Cre plasmid.

The inclusion bodies of TAT-SUMO1 were prepared by sonication of E.coli pellet in solution containing 50 mmol/l Tris, 5 mmol/l EDTA, and 2% Triton X-100 (pH 8.0). Inclusion bodies of TAT-SUMO1 were dissolved in a solution containing 25 mmol/l sodium acetate, 5 mol/l urea, and 1% triton X-100 (pH 4.0) and were loaded onto a SP-Sephadex column (1x10cm, GE Healthcare). The column was washed with a solution containing 25 mmol/l sodium acetate, 0.2 mol/l sodium chloride, 5 mol/l urea, and 1% triton X-100 (pH 4.0) to remove endotoxin from the protein. To remove triton X-100 and urea, the column was subsequently washed with 25 mmol/l sodium acetate and 0.2 mol/l sodium chloride (pH 4.0). Recombinant TAT-SUMO1 was eluted using a solution containing 6 mol/l guanidine-hydrochloric acid and 0.1 mol/l Tris (pH 10.0). Refolding of TAT-SUMO1 was performed by dialyzing against a solution containing 0.5 mol/l arginine, 5 mmol/l cysteine, and 0.5 mmol/l cystine (pH 7.0) for 1 day at 4^{o}C. Finally, refolding solution was exchanged to PBS by dialysis.

TAT-mCherry was extracted with a solution containing 50 mmol/l Tris, 5 mmol/l EDTA, and 1% triton X-100, (pH 8.0) from the E.coli pellet. Ethanol was added to a final concentration of 70% to precipitate the proteins in the extract. TAT-mCherry was dissolved in PBS and then loaded onto a Nickel-NTA column (1x1cm, Qiagen). The column was washed with a solution containing 25 mmol/l Tris and 2% triton X-100 and subsequently washed with a solution containing 25 mmol/l Tris and 50% isopropanol (pH 8.0) to remove endotoxin from the protein. Recombinant TAT-mCherry protein was eluted from the column

using 0.1 mol/l imidazole (pH 8.0). Finally, recombinant TAT-mCherry was dialyzed against PBS.

4.3.c Immunoblotting.

Following treatment, INS 832/13 or human islets were lysed in buffer containing (in mmol/l) 20 Tris-HCl (pH 7.5), 150 NaCl, 1 EGTA, 1 EDTA, 1% Triton-X100, protease inhibitor cocktail (Set V; Millipore), and 25 N-ethylmaleimide. Whole cell lysates were separated using SDS-PAGE, transferred to polyvinylidene difluoride membranes (Millipore), probed with primary antibodies (anti-SUMO1 [PW9460, 1:1000; Enzo Life Sciences]; anti-T7 Tag [13246, 1:1000; Cell Signaling Technology]), and detected with appropriate peroxidase-conjugated secondary antibodies (Amersham). Total protein was assessed using TGX Stain-Free gels (Bio-Rad) and was quantified immediately following transfer to PVDF membrane. Images were acquired using a ChemiDoc MP System (Bio-Rad) and analyzed using Image Lab Software 5.2.1 (Bio-Rad).

4.3.d Nitrite Measurement.

Nitrite accumulation in culture medium was determined by use of Greiss reagent (Sigma), where 50 µl of culture medium was mixed and incubated with 50 µl of Griess reagent for 15 minutes at room temperature. Nitrite concentrations were quantified by measuring absorbance at 540 nm. Dilutions of sodium nitrite were used as standards. Cells were subsequently lysed in in buffer containing (in mmol/l) 20 Tris-HCl (pH 7.5), 150 NaCl, 1 EGTA, 1 EDTA, 1% Triton-X100, protease inhibitor cocktail (Set V; Millipore), and 25 N-

ethylmaleimide and were assayed for protein using a Micro BCA Protein Assay Kit (Thermo Scientific).

4.3.e Electrophysiology.

The standard whole-cell technique with the sine+DC lockin function of an EPC10USB amplifier (HEKA Elektronik) was used to measure cell capacitance in dispersed human islet cells. Experiments were performed at 32-35°C. Bath solution for depolarization trains contained (in mmol/l) 118 NaCl, 20 TEA, 5.6 KCl, 1.2 $MgCl_2 \bullet 6H_2O$, 2.6 $CaCl_2$, 5 glucose, and 5 HEPES (pH 7.4). The pipette solution for depolarization trains contained (in mmol/l) 125 CsOH, 125 glutamate, 10 CsCl, 10 NaCl, 1 $MgCl_2 \bullet 6H_2O$, 0.05 EGTA, 5 HEPES, 0.1 cAMP, and 3 MgATP (pH 7.15). Patch pipettes were pulled from borosilicate glass, were coated with Sylgard, and had resistances of 3-7 MΩ when filled with pipette solution. Whole-cell capacitance responses were normalized to initial cell size. β-cells were positively identified by cell size > 6 pF.

4.3.f Quantitative PCR.

RNA was extracted using TRIzol Reagent (Life Technologies). Real-time quantitative PCR assays were carried out on the 7900HT Fast Real-Time PCR system (Applied Biosystems) using Fast SYBR Green Master Mix (Applied Biosystems) as the amplification system with 300 nmol/l primers and 1-µl template in 20-µl PCR volume and annealing temperature of 60°C. Primers are indicated in Table 5. Results were normalized to *Actb* or *ACTB* expression in rat or human samples, respectively.

4.3.g Statistics.

Data were analyzed using FitMaster (HEKA Elektronik) or GraphPad Prism v6.0c and were compared as indicated in figure legends. Outliers were identified and removed using Grubbs' test for outliers. Data are expressed as means ± SEM, where $P<0.05$ is considered significant.

Figure 25. Amino acid sequence of TAT fusion peptides. ►

Amino acid sequences of TAT-SUMO1 and TAT-mCherry. TAT_{48-57} peptide (green box), T7 tag (grey box), and cargo protein (blue text) indicated. Peptide generation by KS.

Figure 25.

TAT-SUMO1:

MGRKKRRQRRRGHMASMTGGQQMGRDPMSDQEAKPSTEDLGDKKEGE
YIKLKVIGQDSSEIHFKVKMTTHLKKLKESYCQRQGVPMNSLRFLFE
GQRIADNHTPKELGMEEEDVIEVYQEQTGGHSTV

TAT-mCherry:

MGRKKRRQRRRGHMASMTGGQQMGRDPHMVSKGEEDNMAIIKEFMRF
KVHMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFAWDIL
SPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGVVTVT
QDSSLQDGEFIYKVKLRGTNFPSDGPVMQKKTMGWEASSERMYPEDG
ALKGEIKQRLKLKDGGHYDAEVKTTYKAKKPVQLPGAYNVNIKLDIT
SHNEDYTIVEQYERAEGRHSTGGMDELYKLEHHHHHH

Table 5. Primers.

gene	sequence	
Actb	F	5'-TGAAGTGTGACGTTGACATCC-3'
	R	5'-ACAGTGAGGCCAGGATAGAGC-3'
Adm	F	5'-GTTTCCATCGCCCTGATGTTATTG-3'
	R	5'-GCTGCTGGACGCTTGTAGTTC-3'
Bip	F	5'-TATTGAAACTGTGGGAGGTG-3'
	R	5'-ACCCAGAAGGTGATTGTCTT-3'
Bnip3	F	5'-GTTTGCATCCAGGAGAGTCC-3'
	R	5'-TCCAGCACCATCACAGAGAG-3'
cfos	F	5'-GGGAGTGGTGAAGACCATGT-3'
	R	5'-GCTGCAGCCATCTTATTCCT-3'
Chop	F	5'-GGAGGTCCTGTCCTCAGATG-3'
	R	5'-AGGTGCTTGTGACCTCTGCT-3'
Cxcl1	F	5'-GCCATCGGTGCAATCTATCT-3'
	R	5'-GCACCCAAACCGAAGTCATA-3'
ACTB	F	5'-GGACTTCGAGCAAGAGATGG-3'
	R	5'-AGCACTGTGTTGGCGTACAG-3'
BNIP3	F	5'-GAATATTTTCCGGCCGACTT-3'
	R	5'-CCCATAGCATTGGAGAGAAAA-3'
CXCL10	F	5'-ATTTTGCTCCCCTCTGGTTT-3'
	R	5'-CCACGTGTTGAGATCATTGC-3'
HIF1A	F	5'-AGGTGGATATGTCTGGGTTG-3'
	R	5'-AAGGACACATTCTGTTTGTTG-3'

4.4 RESULTS

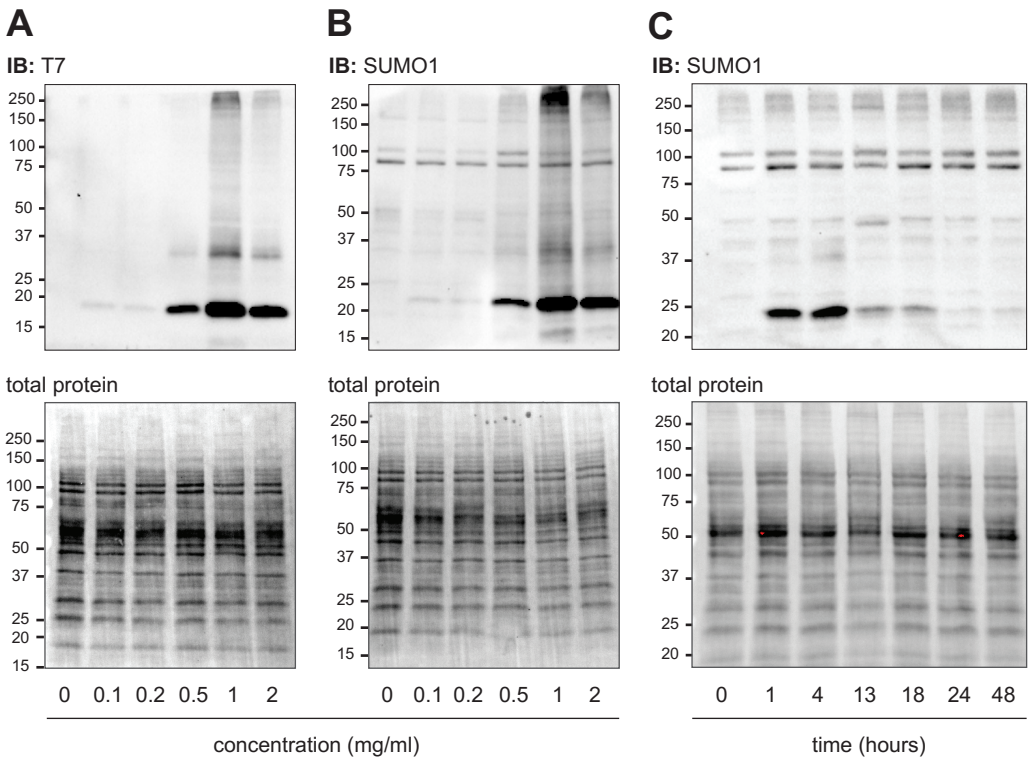
4.4.a TAT-SUMO1 is rapidly transduced into INS 832/13 cells.

To assess whether fusion of a TAT domain to SUMO1 would allow its transduction, INS 832/13 cells were treated with TAT-SUMO1 protein, as indicated, and cellular SUMOylation was assessed. TAT-SUMO1 was transduced into INS 832/13 cells and was highly bioavailable, as indicated by its conjugation to numerous target proteins (Figure 26). Enhanced SUMOylation was due to conjugation of TAT-SUMO1, as opposed to the up-regulation of endogenous SUMO1, as evidenced by enhanced conjugation of the T7 linker tag (Figure 26A). TAT-SUMO1 conjugation was concentration-dependent (Figure 26B). Increased duration of INS 832/13 exposure to TAT-SUMO1 did not result in increased SUMO1 conjugation (Figure 26C) indicating that TAT-SUMO1 is rapidly and efficiently transduced into INS 832/13 cells. Indeed, TAT-mCherry was transduced into isolated human islets within 1-hour of peptide exposure, as observed by the presence of intracellular immunofluorescence (data not shown).

Figure 26. TAT-SUMO1 is cell permeable and bioavailable. ►

SUMO1 conjugation to target proteins assessed by immunoblotting (IB) of SUMO1 in INS 832/13 cell lysates. **A:** Cells were treated with TAT-SUMO1, as indicated, for 18 hours. Total T7 linker conjugation (above) and corresponding levels of total proteins (below) are shown. **B:** Cell treated as in **(B).** Total SUMO1 conjugation (above) and corresponding levels of total proteins (below) are shown. **C:** Cells were treated with TAT-SUMO1 (1 mg/ml), as indicated. Total SUMO1 conjugation (above) and corresponding levels of total proteins (below) are shown. Data are representative of 1 experiment.

Figure 26.


A
IB: T7
250
150
100
75
50
37
25
20
15
total protein
250
150
100
75
50
37
25
20
15
0 0.1 0.2 0.5 1 2
B
IB: SUMO1
250
150
100
75
50
37
25
20
15
total protein
250
150
100
75
50
37
25
20
15
0 0.1 0.2 0.5 1 2
concentration (mg/ml)
C
IB: SUMO1
250
150
100
75
50
37
25
20
total protein
250
150
100
75
50
37
25
20
0 1 4 13 18 24 48
time (hours)

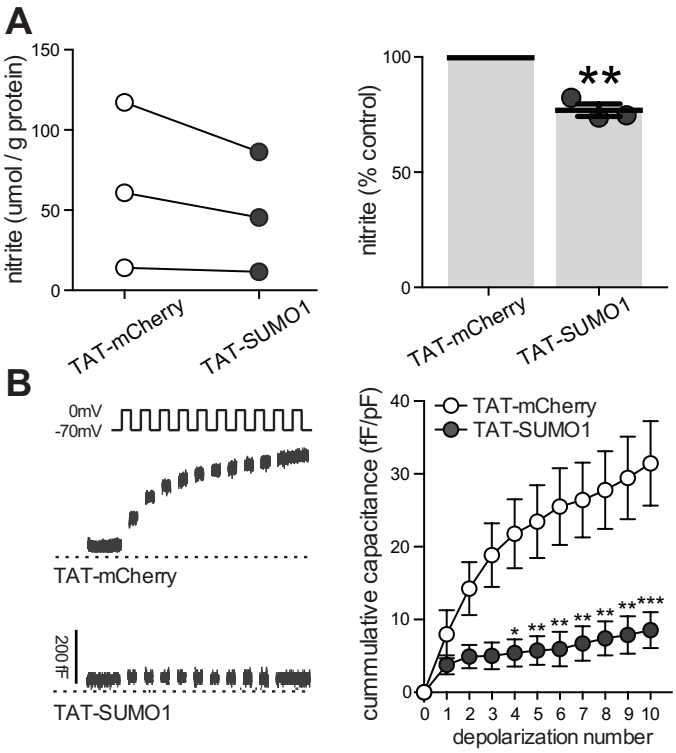
4.4.b Functional effects of SUMO1 are not altered by TAT_{48-57} peptide fusion.

To determine whether fusion of TAT-peptide to SUMO1 would alter its biological activity, the ability of TAT-SUMO1 to replicate previously reported effects of SUMO1 on β-cell function were assessed. Consistent with the action of enhanced SUMOylation on *Nos2* expression (Figure 22), 18-hour pre-treatment of INS 832/13 cells with TAT-SUMO1 (1 mg/ml) significantly reduced IL-1β-induced nitrite production (Figure 27A) compared to TAT-mCherry treated control cells. Nitrite was undetectable in media of unstimulated cells. The biological activity of TAT-SUMO1 was also validated in human β-cells, where SUMO1 inhibits β-cell exocytosis (Dai et al., 2011). Similarly, acute exposure to TAT-SUMO1 (1 mg/ml, 1 hour) reduced depolarization-induced exocytosis in dispersed human β-cells (Figure 27B), indicating that TAT_{48-57} fusion does not alter the biological activity or actions of SUMO1.

Figure 27. Fusion of TAT_{48-57} to SUMO1 does not impair its activity. ►

A: Nitrite accumulation in culture media normalized to protein content (left) and values as a percent of TAT-mCherry control (right) of INS 832/13 cells pre-treated with TAT-SUMO1 or TAT-mCherry (1 mg/ml; 18 hours) and subsequently treated with IL-1β (10 ng/ml; 24 hours) (*n*=3, 3; 3 experiments). **B:** Exocytotic responses of single human β-cells treated with TAT-SUMO1 or TAT-mCherry (1 mg/ml; 1 hour) measured as an increase in cell membrane capacitance during a train of 500 ms depolarizations from -70 to 0 mV (left). Average cumulative capacitance response to each step-wise depolarization (right) (*n*=13, 10 cells; 3 donors); data collection by AB. *n* values correspond to data points from left to right, respectively. Data are mean ± SEM and were compared with **(A)** a two-tailed Student's *t*-test or **(B)** repeated measures ANOVA followed by a Sidak post-test. *P<0.05, **P<0.01, and ***P<0.001 versus TAT-mCherry control.

Figure 27.


A
nitrite (umol / g protein)
150
100
50
0
TAT-mCherry
TAT-SUMO1
nitrite (% control)
100
50
0
**
TAT-mCherry
TAT-SUMO1
B
0mV
-70mV
TAT-mCherry
200fF
TAT-SUMO1
cummulative capacitance (fF/pF)
40
30
20
10
0
TAT-mCherry
TAT-SUMO1
*
**
**
**
**
**
***
0 1 2 3 4 5 6 7 8 9 10
depolarization number

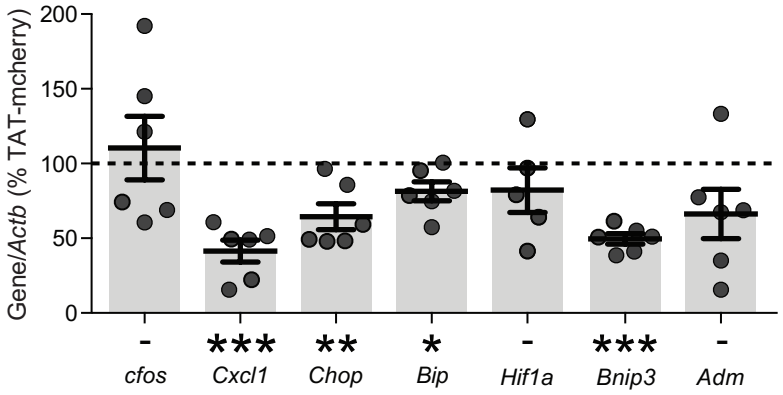
4.4.c TAT-SUMO1 reduces NFκB-, ER-stress, and hypoxia-associated gene transcription in INS 832/13 cells.

Islet isolation activates the inflammation-associated transcription factor NFκB (Abdelli et al., 2004; Bottino et al., 2004) and the pro-apoptotic kinase JNK (Abdelli et al., 2004). INS 832/13 were pre-treated with TAT-SUMO1 or TAT-Cherry (1 mg/ml, 18 hours) and were exposed to IL-1β (10 ng/ml, 24 hours) to induce cellular stress. To examine the effects of SUMOylation on activation of NFκB, HIF1α, and JNK, expression of the NFκB target gene *Cxcl1* (Burke et al., 2014), the HIF1α target genes *Bnip3* and *Adm* (Elvidge et al., 2006), and the JNK downstream target gene *cfos* (Bonny et al., 2001) were quantified. TAT-SUMO1 reduced expression of *Cxcl1* and the pro-apoptotic *Bnip3* (Ray et al., 2000) (Figure 28) indicating that SUMO1 conjugation represses NFκB and HIF1α-regulated transcription. TAT-SUMO1, however, had no effect on transcription of *cfos* (Figure 28). Consistent with the induction of ER stress by SENP1 (Figure 20), expression of the ER stress associated genes *Chop* and *Bip* were also reduced in TAT-SUMO1-treated INS 832/13 cells (Figure 28).

Figure 28. TAT-SUMO1 reduces transcriptional activity of NFκB and HIFα. ►

INS 832/13 cells were pre-treated with TAT-mCherry or TAT-SUMO1 (1 mg/ml; 18 hours) and subsequently treated with IL-1β (10 ng/ml) for 24 hours. RT-PCR detection and quantification of *cfos* (*n*=6, 6), *Cxcl1* (*n*=4, 6), *Chop* (*n*=6, 6), *Bip* (*n*=6, 6), *Hif1a* (*n*=6, 6), *Bnip3* (*n*=6, 6), and *Adm* (*n*=6, 6) expression was determined. Levels of mRNA expression as a percent of TAT-mCherry are shown; data collection and analysis by MF. *n* values correspond to TAT-mCherry and TAT-SUMO1 treatment, respectively, and are representative of 3 experiments. Data are mean ± SEM and were compared with a two-tailed Student's *t*-test. *P<0.05, **P<0.01, and ***P<0.001 versus TAT-mCherry control.

Figure 28.


Gene/Actb (% TAT-mcherry)
200
150
100
50
0
-
***
**
*
-
***
-
cfos
Cxcl1
Chop
Bip
Hif1a
Bnip3
Adm

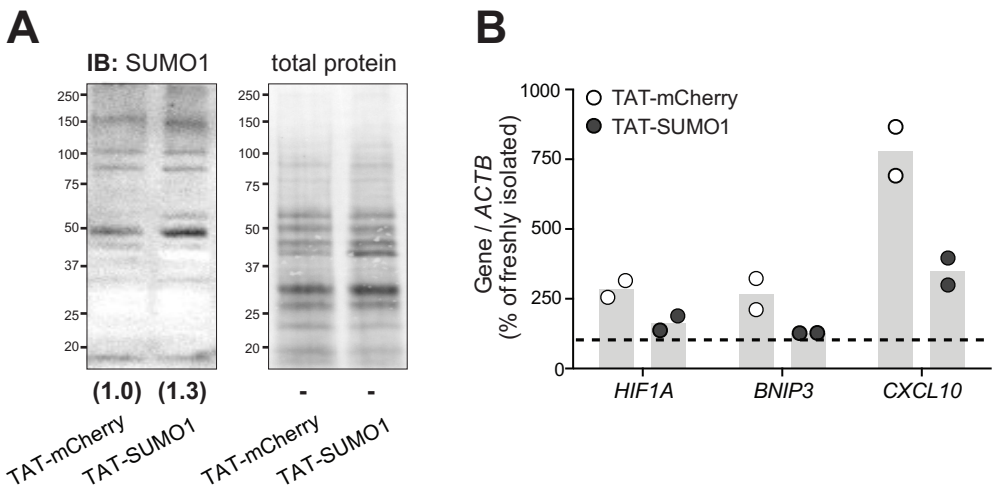
4.4.d TAT-SUMO1 reduces isolation-induced transcription of hypoxia- and inflammation-associated genes.

Given that SUMO1 conjugation reduced activation of NFκB and HIF1α in an insulin-secreting cell line, the ability of TAT-SUMO1 to reduce activation of these pathways following isolation-induced stress was examined. Immediately following isolation, human islets were either collected for analysis or were treated with TAT-mCherry or TAT-SUMO1 (1 mg/ml) for 18 hours. Although TAT-SUMO1 enhanced SUMOylation in human islets (Figure 29A), SUMO1 conjugation in human islets was lower than that observed in INS 8321/13. In spite of the relatively low induction of SUMOylation, treatment with TAT-SUMO1 reduced isolation-induced expression of *HIF1A*, *BNIP3*, and *CXCL10* in islets isolated from one human donor (Figure 29B).

Figure 29. TAT-SUMO1 reduces isolation-induced transcription of hypoxia- and inflammation-associated genes. ►

A: SUMO1 conjugation induced by TAT-SUMO1 or TAT-mCherry treatment (1 mg/ml; 18 hours) assessed by immunoblotting (IB) of SUMO1 in isolated human islets (left) and corresponding levels of total protein (right). Quantification by densitometry relative to TAT-mCherry control is indicated in brackets. **B:** Following isolation, human islets were immediately harvested or were treated with TAT-mCherry or TAT-SUMO1 (1 mg/ml; 18 hours). RT-PCR detection and quantification of *HIF1A* (*n*=2, 2, 2), *BNIP3* (*n*=2, 2, 2), and *CXCL10* (*n*=2, 2, 2) expression was determined. Levels of mRNA expression as a percent of freshly isolated islets are shown and are representative of 1 donor; data collection and analysis by MF. *n* values correspond to freshly isolated islets, TAT-mCherry-, or TAT-SUMO1-treated islets, respectively.

Figure 29.


A
IB: SUMO1
total protein
250
150
100
75
50
37
25
20
(1.0) (1.3)
- -
TAT-mCherry
TAT-SUMO1
TAT-mCherry
TAT-SUMO1
B
TAT-mCherry
TAT-SUMO1
Gene / ACTB
(% of freshly isolated)
1000
750
500
250
0
HIF1A
BNIP3
CXCL10

4.5 DISCUSSION

Islet transplantation has proven successful in reducing erratic blood glucose fluctuations and inducing short-term insulin-independence in T1D patients (Shapiro et al., 2000). However, the inability to consistently isolate a threshold islet mass from a single donor pancreas limits the widespread use of islet transplant therapy. Reducing islet cell death during islet isolation represents an efficient alternative to multi-donor transplantation.

Isolation-induced islet loss has been estimated to reduce islet mass by at least 15% (Bottino et al., 2004). One of the first reported strategies targeting human islet isolation recovery was the use of the antioxidant AEOL10150 (Bottino et al., 2004). Infusion of AEOL10150 during islet isolation markedly repressed NFκB activity, decreased isolation-induced pro-inflammatory cytokine expression, and reduced islet loss by almost 50%. That isolation-induced islet loss still persists despite inhibition of NFκB indicates the presence of NFκB-independent pathways of cell death. The ability to modulate several pathways of islet cell death will be crucial in preserving islet mass during islet isolation.

Here, we demonstrate the ability of SUMO1 conjugation to reduce expression of NFκB target genes in an insulin secreting cell line. These findings implicate SUMO1 as a negative regulator of inflammatory signaling and are consistent with the cytoprotective roles of SUMOylation in other cell types (Hajmrle et al., 2014; Kim et al., 2011; Okura et al., 1996; Pascual et al., 2005). Preliminary evidence indicates that the inhibitory actions of SUMO1 persist in human islet cells and are capable of repressing isolation-induced activation of these pathways. In contrast, up-regulation of SUMO1 conjugation could not reduce transcription of the JNK-activated gene *cfos*. While this suggests that SUMOylation does not regulate JNK, *cfos* transcription is not directly control by JNK and may not accurately reflect its cellular activity.

Under normoxic conditions, HIF1α is continuously degraded by proteasome degradation. When oxygen levels decrease, protein degradation is inhibited, HIF1α binds to HIF1β, and transcription of hypoxia response genes is initiated (Cantley et al., 2010). Although HIF1α is required for β-cell viability during hypoxia (Bensellam et al., 2016), supra-physiological levels of HIFα are cytotoxic to β-cells (Cheng et al., 2010) and are associated with expression of the pro-apoptotic *BNIP3* (Elvidge et al., 2006; Ray et al., 2000). TAT-SUMO1 was found to reduce *BNIP3* expression in both an insulin-secreting cell line and in freshly isolated human islets. While HIF1α activity is primarily regulated through post-translational modifications, NFκB has been implicated as a mediator of HIF1α secondary to regulation of *HIF1A* expression (van Uden et al., 2008). Consistent with its effects on NFκB-mediated transcription, TAT-SUMO1 reduced *HIF1A* expression in human islet cells, raising the possibility that effects of TAT-SUMO1 on *BNIP3* expression may be a consequence of NFκB inhibition rather than direct SUMO1 conjugation to HIF1α.

ER stress is up-regulated in isolated human islets (Negi et al., 2012) as perhaps, a consequence of cytokine-mediated induction of the UPR (Brozzi et al., 2015; Cardozo et al., 2005). SUMO1 up-regulation also reduced expression of ER stress associated genes *Chop* and *Bip* in an insulin-secreting cell line. Whether the actions of SUMO1 on ER stress are direct, or are a consequence of reduced inflammatory signaling, remains unknown. Nevertheless, that SUMO1 conjugation reduced expression of pro-apoptotic *Chop* (Allagnat et al., 2012) supports a cytoprotective role for SUMO1.

Isolated islets are characterized by the up-regulation of several pro-inflammatory cytokines and chemokines (Bottino et al., 2004; Negi et al., 2012; Vargas et al., 1998). Isolation-induced islet inflammation has detrimental consequences to islet transplant outcomes (Moberg et al., 2002): inflammatory markers expressed on isolated islets trigger a

post-transplantation inflammation blood-mediated reaction (IBMR) resulting in impaired islet engraftment (Citro et al., 2013). SUMOylation reduces inflammatory responses and chemokine expression secondary to SUMOylation-mediated peroxisome proliferator-activated receptor γ (PPARγ) translocation (Pascual et al., 2005). Although regulation of PPARγ by SUMO1 was not assessed in the current study, that SUMO1 conjugation reduced expression of the chemokines *Cxcl1* and *CXCL10* demonstrates anti-inflammatory properties of SUMO1.

SUMO1 inhibits insulin secretion by repressing β-cell exocytosis downstream of granule docking. This inhibition occurs rapidly and is easily reversed upon deSUMOylation (Dai et al., 2011). Although inhibition of insulin secretion by SUMO1 appears limiting, it may assist in preserving islet mass. In a process referred to as "β-cell rest," acute inhibition of insulin secretion improves secretory function upon removal of the inhibitory stimulus (Greenwood et al., 1976; Laedtke et al., 2000). Whether this extends to the inhibition of insulin secretion induced by SUMO1 remains unknown. However, that SUMO1 inhibition of insulin secretion is reversible (Dai et al., 2011) and is not associated with enhanced cytotoxicity (Figure 23) indicates that it should not be a factor contraindicating SUMO1 use in the preservation of islet mass prior to transplantation.

For a SUMO1-targeted therapy to be readily adapted for further pre-clinical and clinical studies, a genetic modification-independent transduction strategy must be developed. Several clinical trials examining the efficacy of TAT-conjugated peptides are underway (Rizzuti et al., 2015), with initial studies demonstrating clinical safety and efficacy (Hill et al., 2012). Here, we describe the use of a TAT_{48-57}-mediated transduction strategy to produce a bioavailable small-peptide inducer of SUMOylation, TAT-SUMO1, which rapidly enhances SUMO1 conjugation and produces biologically-appropriate functional effects in

both an insulin secreting cell line and in human islet cells. The effects of TAT-SUMO1 on post-isolation islet mass, function, and inflammatory profiles will be required to determine its utility as an in vitro adjunct therapy for islet transplantation.

4.6 STUDY LIMITATIONS

In the current study, up-regulation of downstream target genes were assessed as indirect measures of NFκB, JNK, and HIF1α activity. Although gene expression is indicative of pathway activation, these findings should be corroborated with direct measures of protein activity and the effects SUMO1 on cell death should be confirmed. Assessment of TUNEL reactivity or caspase 3 cleavage will more accurately reflect the effects of TAT-SUMO1 on apoptosis. Additionally, alternate pathways of cell death should be examined, particularly considering the necrotic-like mechanisms of BNIP3-induced cytotoxicity (Vande Velde et al., 2000). Ultimately, a measure of islet loss, as assessed through direct quantification of preserved islet equivalents, will provide the most relevant measure of TAT-SUMO1 efficacy. Nevertheless, that TAT-SUMO1 reduced expression of pro-apoptotic genes supports a cytoprotective role of this peptide.

SUMO1 conjugation was nominally increased following an 18-hour treatment of freshly isolated human islets with TAT-SUMO1. Further investigation into TAT-SUMO1 transduction and conjugation kinetics in human islets will be required to optimize both the pre-clinical and clinical bio-availability and efficacy of this therapeutic strategy.

4.7 CONCLUDING REMARKS

The current chapter provides preliminary evidence supporting further investigation of TAT-SUMO1 as an adjunct therapy during islet isolation. The pharmacological small-peptide inhibitor strategy proposed avoids the need for genetic modification or viral-mediated transduction strategies rendering the proposed strategy readily adaptable for further pre-clinical and clinical studies. Enhanced conjugation of TAT-SUMO1 resulted in repression of inflammatory and hypoxia-associated gene expression in insulin secreting and human islet cells. These findings support the role of SUMO1 as an anti-apoptotic protein and highlight potential novel pathways of SUMO1 regulation, in particular its ability to reduce hypoxia-associated gene transcription, in insulin secreting cells. Furthermore, the present study has confirmed the production of a cell permeable, bioavailable TAT-SUMO1 peptide. The effects of TAT-SUMO1 on human islet viability and islet graft function are currently underway.

CHAPTER 5.

Summary, discussion, and future directions.

5.1 SUMMARY

5.1.a Literature overview.

The immune system restores homeostasis that is disturbed by injurious stimuli, pathogen invasion, or an abnormal internal environment (Male, 2013). Activation of the immune system comes at a significant metabolic cost, requiring a steady supply of glucose to mount an effective immune response (reviewed in Hotamisligil and Erbay, 2008). Indeed, the immune system has emerged as a critical regulator of energy homeostasis, with impaired immunometabolism contributing to the pathophysiology of T2D (Donath and Shoelson, 2011).

IL-1β plays a critical role in pathogen-sensing, fulfilling adjuvant and pyrogenic functions required for effective immune responses (Glaccum et al., 1997; Kozak et al., 1995; Labow et al., 1997). IL-1β also possesses glucoregulatory actions. Acute up-regulation of IL-1β results in rapid, pronounced hypoglycemia (Besedovsky and del Rey, 1987; Crown et al., 1991; del Rey and Besedovsky, 1987; Oguri et al., 2002). In contrast to its hypoglycemic effects, acute infusion of IL-1β reduces insulin-stimulated glucose uptake in heart, muscle, and liver tissue (Ling et al., 1994). Chronically, the effects of IL-1β on peripheral insulin sensitivity remain inconclusive, with evidence supporting both IL-1-induced insulin resistance (McGillicuddy et al., 2011; Sauter et al., 2008; Vandanmagsar et al., 2011) and IL-1-mediated improvement in insulin sensitivity (García et al., 2006; Matsuki et al., 2003; Somm et al., 2006). In humans, antagonism of IL-1 signaling in T2D or in patients with metabolic syndrome has little effect on insulin sensitivity (Table 2).

Acute infusion of IL-1β is associated with increased plasma insulin concentrations (Cornell, 1989; del Rey and Besedovsky, 1987; Ling et al., 1995), indicative of an IL-1β-mediated potentiation of insulin secretion. Several studies have identified IL-1β as a

regulator of β-cell secretory function, capable of acutely potentiating stimulus-induced insulin secretion from isolated islets (Borg and Eizirik, 1990; Comens et al., 1987; Eizirik and Sandler, 1989; Eizirik et al., 1992; 1993; 1995; Helqvist et al., 1989; Palmer et al., 1989; Welsh et al., 1989; 1991; Zawalich and Diaz, 1986; Zawalich and Zawalich, 1989).

Conversely, chronic exposure to IL-1β is cytotoxic reducing islet secretory responses and viability in both rodent (Mandrup-Poulsen et al., 1986) and human (Rabinovitch et al., 1990) islet cells. IL-1β mediates the detrimental effects of high glucose on human β-cell function and survival (Maedler et al., 2002) implicating IL-1β as a regulator of β-cell failure during T2D, with numerous studies examining the chronic effects of IL-1β on human islets in vitro and in several T2D animals reporting similar cytotoxic effects (Section 1.4.b).

In 2007, a clinical trial examining the effects of IL-1 receptor antagonism in T2D subjects demonstrated modest improvements in glycemia and β-cell secretory function compared to a placebo control (Larsen et al., 2007). While initially promising, several other studies antagonizing this pathway were unable to observe an improvement in glycemia despite effectively reducing markers of IL-1 signaling (Ridker et al., 2012; Rissanen et al., 2012; van Asseldonk et al., 2011; van Poppel et al., 2014). Although relatively overlooked since the mid-1990s, interest in the insulinotropic action of IL-1β was recently renewed (Arous et al., 2015), with a re-emergence of the hypoglycemic effects of IL-1 signaling (del Rey et al., 2006) and studies in *Il1r1*- (García et al., 2006; McGillicuddy et al., 2013) and *Il1rn*- (Matsuki et al., 2003; Somm et al., 2006) deficient mice supporting a positive role for IL-1 signaling in the maintenance of euglycemia.

5.1.b Summary of research findings.

Despite the significant body of work examining the effects of IL-1β on glycemic control, the precise conditions and mechanisms regulating the pro- or anti-diabetic actions of this cytokine remain unknown. In an attempt to provide insight into the pleiotropic effects of IL-1β, the present thesis examined the consequences of IL-1 signaling both in vivo and in vitro, with exploration of the pathways and internal environments regulating its activity. The principle findings of the studies described in the present thesis are summarized below:

- The ability of acute IL-1 signaling to potentiate glucose-stimulated insulin secretion in isolated human and rodent islets was confirmed (Figure 7).

- Using $[Ca^{2+}]_i$ imaging, whole-cell electrophysiology, and electron microscopy, the stimulatory effects of IL-1β were found to be independent of changes in $[Ca^{2+}]_i$ entry and secondary to increased insulin granule docking (Figure 10, Figure 12).

- IL-1β-induced F-actin depolymerization in mouse and human β-cells was observed using immunofluorescence techniques. Potentiation of glucose-stimulated insulin secretion by IL-1β persisted despite complete F-actin depolymerization (Figure 11).

- *Il1r1*-knockout mice were characterized as normoglycemic (Figure 13).

- Islet isolated from *Il1r1*-deficient mice had normal β-cell function (Figure 9) and insulin secretory responses to glucose (Figure 8).

- Mice deficient in *Il1r1* became glucose intolerant subsequent to acute metabolic (Figure 14) or inflammatory (Figure 15) stress.

- Islets from obese donors were sensitized to the stimulatory effects of IL-1β, whereas the insulinotropic effects of this cytokine were absent in islets of obese T2D subjects (Figure 7).

- The cytotoxic effects of chronic IL-1 signaling on β-cell apoptosis, assessed by measurement of caspase 3 cleavage and TUNEL reactivity, were confirmed in an insulin-secreting cell line (Figure 18).

- The cytotoxic effects of IL-1β were enhanced by deSUMOylation induced by adenoviral-mediated overexpression of the SUMO protease SENP1 (Figure 18).

- DeSUMOylation itself was found to reduce viability of human islet cells. The cytotoxic action of SENP1 was associated with transcription of NFκB target genes and was reduced by pharmacological inhibition of NFκB (Figure 21).

- Enhanced SUMOylation, induced by either an adenoviral-mediated up-regulation of SUMO1 or secondary to an siRNA-mediated down-regulation of SENP1, reduced apoptosis in both an insulin secreting cell line and in human islet cells and was protective against IL-1β-induced apoptosis (Figure 22, Figure 23).

- A cell permeable, biologically-active SUMO1 peptide was generated through fusion of SUMO1 to a TAT_{48-57} transduction domain (Figure 26, Figure 27).

- TAT-SUMO1 reduced transcription of several genes associated with inflammation, ER stress, and hypoxia in an IL-1β-treated insulin secreting cell line (Figure 28).

5.2 DISCUSSION

5.2.a The physiological role of IL-1 signaling in glucose homeostasis.

5.2.a.i Evidence that basal IL-1 signaling nominally regulates glucose homeostasis.

Studies examining *Il1r1*-deficient mice have assisted in characterizing the role of basal IL-1 signaling in glucose homeostasis. Although insulin resistance develops in 9 month-old *Il1r1*-deficient mice, glucose intolerance in these animals remains minimal (García et al., 2006; McGillicuddy et al., 2013). The effects of IL-1 signaling on glucose homeostasis have also been examined in *Il1rn*-knockout mice. Consistent with the phenotype of *Il1r1*-deficiency, absence of *Il1rn* reduces body weight, induces hypophagy, and improves insulin sensitivity (Matsuki et al., 2003; Somm et al., 2006).

The findings are in contrast to those of the present studies. Here, absence of *Il1r1* had no effect on glycemia, glucose tolerance, or insulin action in 6 or 9 month-old animals compared to littermate controls (Figure 13). These discrepancies are likely a consequence of procedural differences, with previous studies administering higher doses of glucose (McGillicuddy et al., 2013), or glucose through an intravenous (García et al., 2006)—rather than intraperitoneal (Figure 13)—route. These differences exacerbate nutrient excess and represent a response to acute metabolic stress rather than a physiological change in glucose tolerance. Indeed, differences in glucose tolerance observed in the previous studies only emerged 50 minutes post-glucose stimulation, suggesting that late feedback mechanisms, such as the metabolic stress-induced up-regulation of IL-1β, are underlying the effects observed. Although reports of mild glucose intolerance in aged *Il1r1*-deficient mice suggest that basal IL-1 signaling is primarily anti-diabetic, given the small amplitude of these effects together with their absence in the present study indicates that IL-1 signaling nominally

contributes to the maintenance of euglycemia under basal, non-pathophysiological conditions.

5.2.a.i Evidence that IL-1 signaling is required for the maintenance of euglycemia in response to acute metabolic stress.

To characterize the role of IL-1 signaling on β-cell function, islets were isolated from *Il1r1*-knockout mice and several aspects of β-cell function and secretory responses to glucose were assessed. Although a slight impairment in the ability of glucose to depolymerize F-actin was observed in *Il1r1*-deficient islets (Figure 9), this did not result in significant reductions in granule recruitment, exocytosis, or glucose-stimulated insulin secretion (Figure 8). This is consistent with isolated human islets, where inhibition of IL-1 signaling does not affect secretory responses to glucose (Giannoukakis et al., 1999; Maedler et al., 2002). IL-1 receptors, however, are abundantly expressed on pancreatic β-cells (Böni-Schnetzler et al., 2009), suggestive of an IL-1-mediated regulation of β-cell function.

In the present study, *Il1r1*-deficient mice fed a HFD for 2 days had impaired glucose tolerance compared to littermate controls (Figure 14A) demonstrating the importance of IL-1 signaling in the maintenance of euglycemia following acute bouts of overnutrition. As discussed previously (Section 2.6), due to the use of whole-body knockout animals, the contribution of β-cell-independent effects to the glucose intolerance observed cannot be excluded. However, that secretory function was impaired in *Il1r1*-knockout mice (Figure 14C) and that insulin tolerance appears normal or even slightly improved (Figure 16) indicates that an inability of *Il1r1*-deficient β-cells to adequately up-regulate insulin secretion underlies the glucose intolerance observed.

While IL-1 signaling emerges as a regulator of glucose homeostasis under conditions of metabolic stress, consequences of IL-1 signaling are dependent on the duration of overnutrition (Table 6). In contrast to the present study, *Il1r1*-deficient mice fed a HFD for 3 months have reduced fasting glucose, improved glucose tolerance, and enhanced secretory function (McGillicuddy et al., 2011). Following 6 months of HFD, however, *Il1r1*-knockout mice return to glucose intolerance (McGillicuddy et al., 2013). Impaired glycemia in these animals is associated with reduced insulin sensitivity and occurs in spite of improved in vivo β-cell secretory function (McGillicuddy et al., 2013). Further study will be required to confirm the relative contribution of the insulinotropic and insulin sensitizing actions of IL-1β in the maintenance of euglycemia during both acute and chronic metabolic stress.

5.2.a.iii Evidence that central actions of IL-1 signaling may be differentially regulated by inflammatory and metabolic stress.

The hypoglycemic effects IL-1β are attributed, in part, to the modulation of the central homeostatic "set-point" of glucose (del Rey et al., 2006). This is evidenced by findings that central inhibition of IL-1 signaling reduces IL-1β-induced hypoglycemia, and that IL-1β-induced hypoglycemia is maintained despite administration of exogenous glucose. The current study examined the role of IL-1 signaling on glucose homeostasis following acute inflammatory stress. Consistent with the central role of IL-1 as a regulator of the glucose homeostatic "set-point," fasting blood glucose levels were reduced in wild-type versus *Il1r1*-knockout mice following LPS treatment (Figure 15B). In contrast, no differences in fasting glucose concentrations were observed following exposure to acute metabolic stress (Figure 14B). Together, these data suggest that the central effects of IL-1β

are differentially regulated by acute inflammatory or metabolic stress. Although the concentrations of IL-1β following HFD or LPS were not assessed in the current study, that IL-1β concentrations are higher during sepsis (Gentile et al., 2013) than obesity (El-Wakkad et al., 2013; Misaki et al., 2010) indicates that IL-1β up-regulation during metabolic stress may not be as pronounced as the up-regulation induced by endotoxins. The centrally-mediated induction of hypoglycemia, therefore, may require elevated concentrations of IL-1β or perhaps, a particular inflammatory profile that is not produced subsequent to nutrient excess. Whether the effects of IL-1β on glucose homeostasis are concentration-dependent, however, remains unknown.

Table 6. Effects of HFD on glucose homeostasis in *Il1r1*-deficient mice.

HFD duration	fasting glucose	glucose tolerance	insulin sensitivity	secretory response	source
2 days	=	↓↓	=	↓	Figure 14
9 days	=	↓	=	↓	
30 days	=	=	ND	=	
3 months	↓	↑↑	↑	↑	McGillicuddy, 2011
4 months	↓	ND	ND	ND	deRoos, 2009
6 months	↑	↓↓	↓	↑	McGillicuddy, 2013

Glycemic parameters of *Il1r1*-deficient mice fed high fat diet for 2 days to 6 months versus HFD fed wild-type controls. ND: not determined.

5.2.b The insulinotropic actions of IL-1β.

5.2.b.i Evidence that IL-1 signaling contributes to islet compensation.

Although obesity is a potent environmental risk factors for the development of T2D (Chan et al., 1994; Colditz et al., 1990), not all obese patients become glucose intolerant. Islets from obese non-diabetic donors possess attributes that render them resistant to the diabetogenic actions of nutrient excess (Prentki and Nolan, 2006). In the present studies, islets from obese donors were found to be sensitized to the insulinotropic effects of IL-1β—a process that was correlated with islet donor BMI (Figure 7). That IL-1β action was increased in islets considered well-adapted to metabolic stress, together with its role in the maintenance of euglycemia under conditions of acute metabolic stress (Figure 14), implicates IL-1 signaling as a positive regulator of islet compensation.

5.2.b.ii Potential mechanisms underlying sensitization of islets from obese donors to IL-1β.

The ability of IL-1β to potentiate insulin secretion was not correlated to the stimulation index of glucose (Figure 7) suggesting that an overall enhancement of secretory function is not underlying these effects. Although mechanisms sensitizing islets to IL-1 signaling remain unknown, several potential regulatory sites are proposed:

RECEPTOR EXPRESSION. *IL1R1* expression is increased in adipose tissue of obese subjects compared to lean controls (Juge-Aubry et al., 2004) suggesting that a metabolic stress-mediated up-regulation of islet *IL1R1* expression may underlie the sensitization of islets from obese donors to IL-1β. Quantification of *IL1R1* expression in islets from lean and obese donors will assist in elucidating the role of receptor up-regulation in this process.

RECEPTOR ACTIVATION. Alterations in IL-1β sensitivity may be mediated by obesity-induced changes to receptor signaling. Following stimulation, IL-1R1 translocates to lipid rafts, which is required for IL-1β-induced activation of NFκB (Oakley et al., 2009). That lipid raft composition is functionally altered in response to nutrient stimulation (Stulnig et al., 2001) suggests that nutrient-mediated modulation of receptor activation may underlie enhanced sensitivity to IL-1β.

POST-RECEPTOR SIGNALING. Differences in sensitivity may also be mediated by post-receptor modulation of intracellular signaling pathways. Investigation of this as a contributory process will require elucidation of the molecular mechanisms regulating the insulinotropic actions IL-1β—a mechanism that is currently unknown.

5.2.b.iii Evidence that reduced IL-1 signaling contributes to β-cell maladaptation.

In support of IL-1 signaling as a regulator of islet compensation to metabolic stress, IL-1β was unable potentiate glucose-stimulated insulin secretion in islets from obese T2D donors. Although a reduction in the overall viability or function of T2D islets may be responsible for the effects observed, that dysfunction in T2D islets is surmountable (Del Guerra et al., 2005; Ferdaoussi et al., 2015; Marchetti et al., 2004) and that secretory function can be potentiated by certain insulinotropic stimuli (Figure 31) suggests that dysfunction observed in the current study is stimulus-dependent. Whether the function of the particular islets used in the present study could be restored, however, was not assessed. Further exploration of the insulinotropic actions in T2D islets will determine whether the impaired response of obese T2D islets to the insulinotropic actions of IL-1β persists across larger sample sizes, whether this also occurs in lean T2D donors, and whether the impaired response is specific to IL-1β.

5.2.b.iv Implications of enhanced sensitivity to acute IL-1 signaling in islets of obese non-diabetic donors.

Enhanced sensitization to insulinotropic effects of IL-1β in obese non-diabetic donor islets suggests that these islets may also be sensitized to the cytotoxic effects of this cytokine. However, that these subjects remain non-diabetic indicates the presence of cytoprotective mechanisms capable of negating the detrimental effects of IL-1β. Indeed, islet responses to the cytotoxic effects of IL-1β varies between islet donors, as evidenced by the discrepant results regarding IL-1β-induced cytotoxicity (Eizirik et al., 1993; Maedler et al., 2002; Rabinovitch et al., 1990). Potential mechanisms dissociating the insulinotropic and cytotoxic effects are discussed below (and are summarized in Figure 30):

DISTINCT SIGNALING PATHWAYS. Mechanisms underlying the insulinotropic actions of IL-1β are unknown and may be distinct to those mediating IL-1β-induced cytotoxicity. Activation of pathway(s) mediating the insulinotropic effects of IL-1β may be favored or an activation of NFκB may be dampened in islet of obese non-diabetic subjects. Investigation of this model as a mediator of compensation will require elucidation of the molecular mechanisms regulating the insulinotropic actions IL-1β.

CYTOPROTECTIVE GENE TRANSCRIPTION. NFκB has been implicated as a mediator of the insulinotropic effects of IL-1β (Section 5.2.b.vi). Although NFκB is associated with IL-1β-induced cytotoxicity, it is also a regulator of several cytoprotective proteins including mitochondrial superoxide dismutase (MnSOD), 70 kilodalton heat shock protein (HSP70) (Cardozo et al., 2001), and TNFα-induced protein 3 (A20) (Liuwantara et al., 2006). NFκB is required for optimal β-cell function (Norlin et al., 2005) and its inhibition has been associated with improved β-cell viability in response to certain death stimuli (Chang et al., 2003). Up-

regulation of cytoprotective gene expression may suppress the cytotoxic effects of NFκB in islet of obese non-diabetic donors. In support of this, T2D islets are characterized by reduced MnSOD transcript expression (Marchetti et al., 2004) and enhanced oxidative stress (Del Guerra et al., 2005). Quantification of IL-1-induced cytoprotective gene expression in islets from obese non-diabetic and T2D donors will assist in elucidating the role of this process in islet compensation.

FEEDBACK INHIBITION. Temporal mechanisms regulating NFκB activity may also underlie the compensation observed. High glucose induces *Sumo1* expression in mouse islets (Rajan et al., 2012) and is associated with up-regulation of SUMO1 conjugation to target proteins (Dai et al., 2011; Figure 30D). SUMO1 inhibits NFκB activity (Desterro et al., 1998). Up-regulation of SUMOylation subsequent to glucose stimulation may reduce NFκB-mediated gene transcription prior to the induction of pro-apoptotic gene expression. Whether SUMO1-mediated regulation of NFκB is altered under conditions of obesity or T2D, however, remains unknown.

5.2.b.v Evidence that IL-1β potentiates glucose-stimulated insulin secretion secondary to enhanced insulin granule recruitment and docking.

In 2015, a study published by Arous and colleagues provided novel insights into the effects of acute IL-1 signaling on β-cell function. In addition to confirming the insulinotropic actions of this cytokine, they proposed that the effects of IL-1β were secondary to activation of FA-associated proteins (Arous et al., 2015). In spite of this, the role of $[Ca^{2+}]_i$, and the actin cytoskeleton in this process remained inconclusive. In the present study, the actions of IL-1β-mediated potentiation of insulin secretion, at least in part, were localized to sites

downstream of $[Ca^{2+}]_i$, entry (Figure 10). Consistent with effects of IL-1β on cytoskeletal rearrangement in rat β-cells (Arous et al., 2015), IL-1β induced cortical F-actin depolymerization in both human and mouse β-cells (Figure 11). While this was associated with increased granule recruitment and exocytosis (Figure 12), the insulinotropic actions of IL-1β persisted in the presence of complete F-actin depolymerization (Figure 11) suggesting the presence of F-actin-independent effects. Indeed, IL-1β was found to enhance granule density within 200 nm of the plasma membrane and increase SNARE complex formation (Figure 12). Together, these results demonstrate that IL-1β potentiates stimulus-induced insulin secretion secondary to enhanced insulin granule recruitment and docking.

5.2.b.vi Potential molecular mechanisms mediating the insulinotropic effects of IL-1β.

Elucidating the molecular mechanisms underlying IL-1β-mediated potentiation of insulin secretion is crucial to understanding the pleiotropic consequences of IL-1 signaling. While mechanisms regulating this process remain unknown, evidence has implicated both NFκB and protein kinase C (PKC) as mediators of the insulinotropic actions of IL-1β:

NUCLEAR FACTOR κB. The implication of NFκB as mediator of IL-1-potentiation of stimulus-induced insulin secretion is based on findings that NFκB is rapidly activated by IL-1β (Figure 23D) and that the insulinotropic effects of this cytokine are inhibited by two NFκB inhibitors, PDTC (Figure 32A) and BAY 11-7082 (Arous et al., 2015). As with most pharmacological inhibitors, however, PDTC and BAY 11-7082 are characterized by several off-target effects (Hartsfield et al., 1998; Rauert-Wunderlich et al., 2013) confounding the results of these studies. The phosphorylation-resistant mutant of IκBα functions as a constitutive repressor of NFκB activity (Brockman et al., 1995). Mutant IκBα is not degraded in response to IL-1

signaling (Figure 32B). Consequently, NFκB is retained in the cytosol and IL-1β-induced NFκB-mediated gene transcription is inhibited. Given the specificity of this strategy for NFκB signaling, overexpression of the IκBα mutant will assist in confirming the role of NFκB in the insulinotropic actions of IL-1β.

PROTEIN KINASE C. PKC belongs to a family of serine/threonine kinases that are activated by the second messenger diacylglycerol (Wang, 2006). PKC activation has been implicated in vesicle recruitment in neurons (Nagy et al., 2002) and enhances Ca^{2+}-stimulated exocytosis in insulin secreting cells (Mendez et al., 2003). PKC also activates protein kinase D (Zugaza et al., 1996)—a known regulator of cortical F-actin depolymerization in pancreatic β-cells (Ferdaoussi et al., 2012). PKC activation has been implicated in acute IL-1 signaling: IL-1β rapidly up-regulates diacylglycerol formation, enhances PKC substrate phosphorylation, and pharmacological inhibition of PKC inhibits the insulinotropic actions of IL-1β (Eizirik et al., 1995). Given the potential effects of PKC on both granule recruitment and F-actin remodeling, further investigation into the role of PKC as a mediator of the insulinotropic actions of IL-1β is warranted.

Figure 30. Potential mechanisms dissociating the insulinotropic and cytotoxic effects of IL-1β. ►

A: Activation of an NFκB-independent insulinotropic pathway may predominate in islets of obese non-diabetic, but not T2D, donors. **B:** NFκB-mediated transcription of cytoprotective genes may be up-regulated in obese non-diabetic, but not T2D, islets. **C:** Glucose-induced up-regulation of SUMOylation may inhibit NFκB-induced transcription of pro-apoptotic genes in obese non-diabetic, but not T2D, islets. **D:** Glucose regulation of SUMO1 conjugation to synaptotagmin VII is duration-dependent. INS 832/13 cells were stimulated with glucose (in mmol/l), as indicated. Immunoprecipitation of synaptotagmin VII followed by immunoblotting for SUMO1 is shown. Reduction in SUMO1 conjugation by glucose stimulation is followed by enhanced SUMO1 conjugation (indicated by star). Panel D adapted with permission from Dai et al., 2011.

Figure 30.

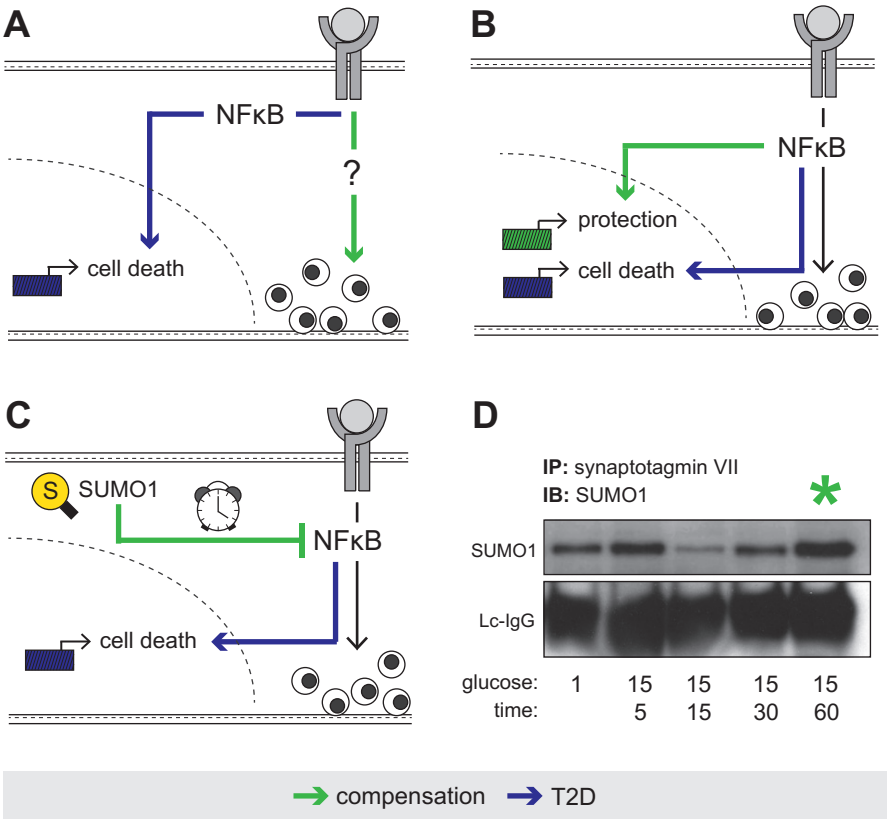

A
NFκB
?
cell death
B
NFκB
protection
cell death
C
S
SUMO1
NFκB
cell death
D
IP: synaptotagmin VII
IB: SUMO1
SUMO1
Lc-IgG
glucose: 1 15 15 15 15
time: 5 15 30 60
compensation
T2D

Figure 31. IBMX potentiates glucose-stimulated insulin secretion in islet from T2D donors. ►

A: Insulin secretion normalized to percent insulin content from T2D human islets following exposure to IL-1β (10 ng/ml, 1 hour), as indicated, and stimulated with 1.0 or 16.7 mmol/l glucose (*n*=9, 9, 9, 9; 3 donors). Insulin contents are shown (inset). **B:** As in **(A)** but treated with 3-isobutyl-1-methylxanthine (IBMX; 100 μmol/l, 2 hours), as indicated (*n*=8, 9, 9, 9; 3 donors); data collection by JK. **C:** Ratio of insulin secretion in response to IBMX treatment, as in **(B)**, in the presence of 16.7 mmol/l glucose to insulin secretion induced by 16.7 mmol/l glucose alone (potentiation index) in islets from non-diabetic (ND) and T2D subjects (*n*=3, 4 donors). Insulin secretion assessed using methods described in Section 2.3a and 2.3b, unless otherwise indicated. *n* values correspond to data points from left to right. Data are mean ± SEM.

Figure 31.

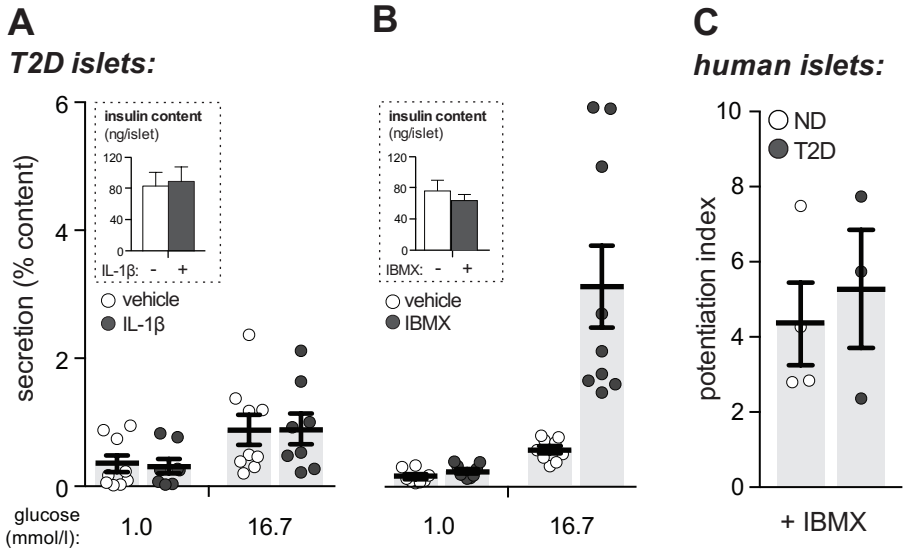
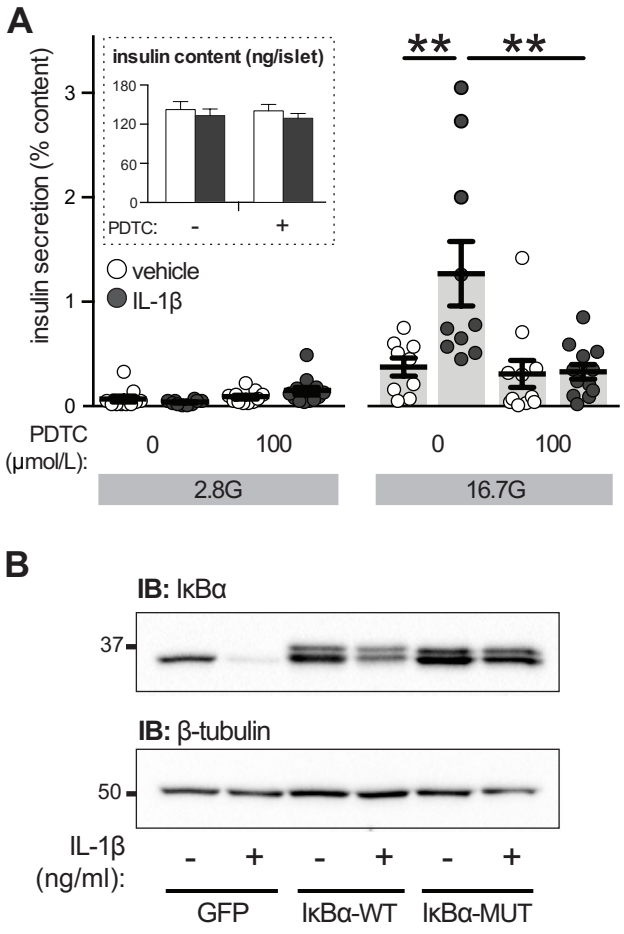

A
T2D islets:
secretion (% content)
insulin content
(ng/islet)
IL-1β: - +
vehicle
IL-1β
glucose
(mmol/l):
1.0
16.7
B
insulin content
(ng/islet)
IBMX: - +
vehicle
IBMX
1.0
16.7
C
human islets:
ND
T2D
potentiation index
+ IBMX

Figure 32. The insulinotropic actions of IL-1β are inhibited by PDTC. ►

A: Insulin secretion normalized to percent insulin content from mouse islets following exposure to the NFκB inhibitor PDTC (100 μmol/l, 5 hour) and to IL-1β (10 ng/ml, 4 hour), as indicated, and stimulated with 2.8 or 16.7 mmol/l glucose (*n*=11, 10, 11, 12, 9, 10, 11, 12; 4 experiments). Insulin contents are shown (inset); data collection by AFS. Insulin secretion assessed using methods described in Section 2.3.a and 2.3.b, unless otherwise indicated. *n* values correspond to data points from left to right. Data are mean ± SEM and compared with 2-way ANOVA followed by Tukey post-test. ***P*<0.01 as indicated. **B:** INS 832/13 cells were transfected with GFP, wild-type IκBα (IκBα-WT), or the phosphorylation mutant form of IκBα (IκBα-MUT) and treated with IL-1β (10 ng/ml, 30 minutes). Transfection and protocols described in Section 3.3.b, unless otherwise indicated. IκBα and β-tubulin expression were assessed by immunoblotting methods described in Section 3.3.f, unless otherwise indicated.

Figure 32.


A
insulin secretion (% content)
insulin content (ng/islet)
180
120
60
0
PDTC: - +
vehicle
IL-1β
3
2
1
0
**
**
PDTC (μmol/L):
0
100
0
100
2.8G
16.7G
B
IB: IκBα
37
IB: β-tubulin
50
IL-1β (ng/ml):
- + - + - +
GFP
IκBα-WT
IκBα-MUT

5.2.c Regulation of IL-1 signaling by SUMOylation.

5.2.c.i Evidence that SUMOylation regulates IL-1 signaling.

SUMO1 is increased following high glucose stimulation of rat mesangial cells (Huang et al., 2013) and transcript expression of *Sumo1* is up-regulated in mouse islets exposed to high glucose (Rajan et al., 2012). That SUMO1 expression is modulated by glucose stimulation suggests that β-cell SUMOylation may be disproportionately up-regulated under conditions of T2D-associated hyperglycemia (Table 1). Consistent with this, secretory dysfunction in β-cells of T2D donors is circumvented following acute infusion of the deSUMOylation enzyme SENP1 or by metabolic co-factors known to induce SENP1 activity (Ferdaoussi et al., 2015). SUMO1 is a negative regulator of NFκB (Akar and Feinstein, 2009; Desterro et al., 1998; Liu et al., 2009) and JNK (Kim et al., 2011) signaling in other cell types. Given the importance of these pathways in regulating IL-1β-induced cytotoxicity and its implication in T2D pathophysiology, the ability of SUMOylation to regulate IL-1 signaling was investigated.

In the current studies, enhanced SUMOylation was induced (1) by the adenoviral-mediated over-expression of SUMO1 (Figure 23); (2) secondary to the siRNA-mediated down-regulation of the deSUMOylating enzyme SENP1 (Figure 22); or (3) by treatment of cells with the TAT_{48-57} fusion peptide (Figure 26). Up-regulation of SUMO1 conjugation reduced IL-1β-induced apoptosis (Figure 23) and decreased expression of genes associated with β-cell death (Figure 28). While the target(s) of SUMO1 conjugation were not determined in the present studies, potential SUMOylation targets capable of modulating IL-1 signaling are discussed below:

NUCLEAR FACTOR κB. The cytoprotective effects of SUMO1 against IL-1β-mediated apoptosis were associated with reduced nuclear translocation of NFκB (Figure 23) and transcription of the NFκB target genes *Nos2* (Figure 22) and *Cxcl1* (Figure 28), implicating SUMO1 as a regulator IL-1β-induced NFκB activity. Indeed, SENP1-mediated potentiation of IL-1β cytotoxicity occurred concomitantly with increased *Nos2* and iNOS expression (Figure 20), suggestive of a deSUMOylation-mediated activation of NFκB. The use of specific NFκB inhibitors, such as the IκBα phosphorylation mutant (Figure 32B; Brockman et al., 1995), will assist in confirming the involvement the NFκB pathway in SUMO1-mediated regulation of IL-1 signaling.

c-JUN NH_2-TERMINAL KINASE. JNK belongs to the mitogen activated protein kinase (MAPK) class of stress kinases that are activated subsequent to stimulus-mediated induction of the MAPK phosphorylation cascade (reviewed in Arthur and Ley, 2013). IL-1β activates JNK in human pancreatic islets (Abdelli et al., 2004; Fornoni et al., 2008) and is hypothesized to induce β-cell apoptosis through inhibition of the anti-apoptotic proteins B-cell lymphoma (Bcl)-2 and Bcl-xL (Cnop et al., 2005) and secondary to activation of pro-apoptotic transcription factors (Davis, 2000). That SUMO1 overexpression reduces heat-shock induced activation of JNK (Kim et al., 2011) suggests that the effects of SUMOylation on IL-1β-induced apoptosis may involve JNK inhibition. However, up-regulation of SUMOylation by TAT-SUMO1 had no effect on *cfos* (Figure 28)—a downstream target gene of JNK.

REDUCED EXPRESSION OF INFLAMMATORY GENES. While there is conflicting evidence surrounding the sufficiency of IL-1β in inducing β-cell apoptosis, that IL-1β cytotoxicity is enhanced in inflammatory environments is well-accepted (Cnop et al., 2005). Exposure of β-cells in vitro to a combination of pro-inflammatory cytokines enhances IL-1β cytotoxicity

(Eizirik et al., 1994; Rabinovitch et al., 1990). Indeed, although IL-1β alone is not associated with diabetes risk, subjects with elevated levels of both IL-6 and IL-1β at greater risk for type 2 diabetes (Spranger et al., 2003). IL-1R antagonism protects against β-cell dysfunction in obese mice expressing the inflammatory peptide hIAPP, but not in obese wild-type mice (Westwell-Roper et al., 2015), demonstrating that inflammatory milieus also sensitize β-cells to the cytotoxic actions of IL-1β in vivo. SUMOylation is known to reduce expression of inflammatory genes by enhancing nuclear translocation of PPARγ (Pascual et al., 2005). SUMO1, therefore, may indirectly reduce the cytotoxic effects of IL-1β by abrogating the inflammatory milieu permissive to IL-1β-induced cytotoxicity. Although regulation of PPARγ by SUMO1 was not assessed in the current study, that SUMO1 conjugation reduced expression of the chemokines *Cxcl1* (Figure 28) and *CXCL10* (Figure 29) implicates SUMO1 as a negative regulator of islet inflammation.

While the present studies establish SUMO1 as a regulator of *persistent* IL-1 signaling, whether SUMOylation regulates the *acute* insulinotropic effects of IL-1β remains unknown. Investigation of SUMO1 as a mediator of cytotoxic IL-1 signaling in human islets was precluded in the current study due to the ineffective induction of apoptosis by IL-1β alone. However, given the potent effects of *acute* IL-1 signaling on human β-cell function, examination of the regulatory role of SUMO1 should be possible in this context.

5.2.c.iii Implications for the regulation of IL-1 signaling by SUMOylation in type 2 diabetes.

The studies described in the present thesis have demonstrated that insulinotropic effects of IL-1β are enhanced in islets from obese, but not T2D, donors (Chapter 2), and that persistent IL-1 signaling is potentiated by SENP1 (Chapter 3). While these findings require

confirmation in human β-cells, as well as in the context of acute IL-1 signaling, a model implicating a role for SUMO1 in the sensitization of islets from obese donors to the insulinotropic actions of IL-1β is proposed (and summarized in Figure 33):

LEAN SUBJECTS. Although IL-1 signaling may or may not potentiate glucose-stimulated insulin secretion in lean subjects (Table 3), IL-1 signaling is not required for the maintenance of euglycemia in this condition (Figure 13).

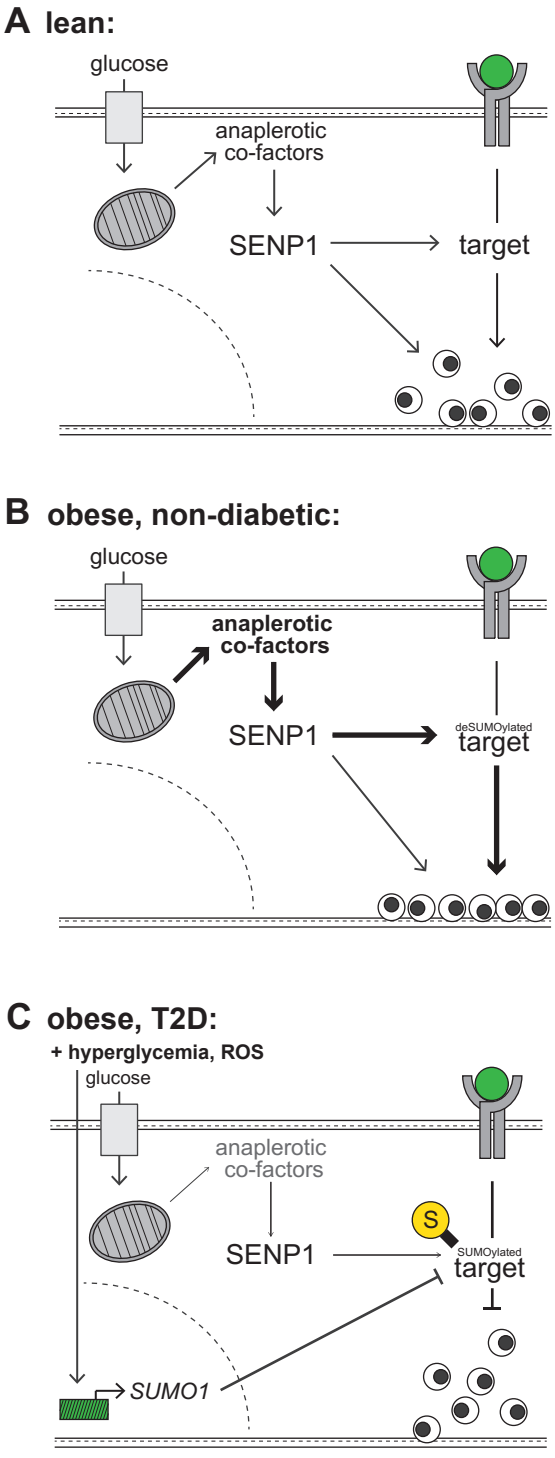
OBESE, COMPENSATED SUBJECTS. In addition to inducing insulin secretion through the triggering pathway, glucose flux through anaplerosis results in the production of metabolic co-factors that potentiate insulin release through a process referred to as the "amplifying" pathway (reviewed in Henquin, 2000). Compensated islets are characterized by enhanced glucose flux through anaplerotic pathways (Liu et al., 2002; Prentki and Nolan, 2006). Co-factors derived from anaplerosis activate SENP1 (Ferdaoussi et al., 2015) suggesting that SENP1 activity may be enhanced in islets of non-diabetic obese donors. Increased SENP1 activity sensitizes β-cells to acute IL-1 signaling (Figure 18) and may underlie the IL-1β-induced potentiation of glucose-stimulated insulin secretion in these islets.

TYPE 2 DIABETIC SUBJECTS. Hyperglycemia, characteristic of T2D subjects (Table 1, Table 3), is known to induce up-regulation of *Sumo1* in pancreatic islets (Rajan et al., 2012). In contrast to islets from obese non-diabetic donors, basal SUMO1 expression may be increased in T2D islets. Enhanced SUMOylation reduces IL-1 signaling (Figure 23), which may contribute to the impaired IL-1β-mediated potentiation of glucose-stimulated insulin secretion observed in these cells (Figure 7).

Figure 33. Proposed model for the role of SUMO1 in the sensitization of islets of obese donors to the insulinotropic actions of IL-1β. ►

A: Acute IL-1 signaling has nominal effects in islets of lean donors. **B:** Enhanced generation of anaplerotic co-factors, characteristic of compensated islets, induces activation SENP1, which may sensitize islets of obese non-diabetic donors to IL-1β. **C:** Hyperglycemia, characteristic of T2D subjects, may up-regulate *SUMO1* expression, resulting in inhibition of target proteins which mediate the insulinotropic actions of IL-1β.

Figure 33.


A lean:
glucose
anaplerotic
co-factors
SENP1
target
B obese, non-diabetic:
glucose
anaplerotic
co-factors
SENP1
deSUMOylated
target
C obese, T2D:
+ hyperglycemia, ROS
glucose
anaplerotic
co-factors
S
SENP1
SUMOylated
target
SUMO1

5.2.c SUMO1 and β-cell survival.

5.2.c.i Evidence that SUMO1 conjugation regulates β-cell viability through modulation of NFκB activity.

The current studies have identified SUMO1 as a novel regulator of pancreatic β-cell viability (Figure 22, Figure 23). SUMO1 regulation of the NFκB cascade occurs at several levels, including regulation of IKK activity (Huang et al., 2003), processing of NFκB subunits (Vatsyayan et al., 2008), and degradation of IκBα (Desterro et al., 1998). SUMOylation of these targets are stimuli-dependent and result in pleiotropic effects on NFκB activity: SUMOylation of IKK is induced by genotoxic stress and enhances NFκB activity (Huang et al., 2003), while regulation of NFκB subunit processing results in inhibition of non-canonical NFκB signaling (Vatsyayan et al., 2008). Consistent with the effects of IκBα SUMOylation on NFκB activity (Desterro et al., 1998), the inhibitory effects of SUMO1 in the present study were associated with reduced nuclear translocation of canonical NFκB (Figure 23) and resulted in an inhibition of NFκB-regulated gene transcription (Figure 28).

SUMO3 is also expressed in insulin secreting cells and in primary human islets (Dai et al., 2009). In parallel to SUMO1, the effects of SUMO3 on NFκB activity are pleiotropic. SUMO3 conjugation enhances NFκB activity secondary to potentiation of IκBα degradation (Aillet et al., 2012) and inhibits NFκB activity through direct conjugation to NFκB (Liu et al., 2012). That SUMO3 regulates NFκB activity downstream of IκBα warrants further exploration of this isoform in the regulation of β-cell viability.

5.2.c.ii Evidence that SENP1-induced secretory dysfunction is secondary to the induction of β-cell death.

SENP1 is required for the amplification of insulin secretion by glucose (Ferdaoussi et al., 2015). In contrast, studies presented here demonstrate that over-expression of SENP1 impairs insulin secretion secondary to enhanced β-cell apoptosis (Figure 21). Together, these findings indicate a spectrum of SENP1 action: while SENP1 is required for glucose-induced insulin secretion, supra-physiological concentrations of SENP1 promote β-cell death. Evidence from islet-specific *Senp1*-knockout mice demonstrates that cytotoxic concentrations of SENP1 are not attained under physiological conditions, as islet morphology and β-cell mass are unchanged in these mice (Ferdaoussi et al., 2015). Recently, acute SENP1 infusion was found to restore exocytotic function to T2D β-cells. In light of these findings, the SENP1 pathway may be an attractive approach to enhance insulin secretion in T2D patients. However, it is important to consider the potential detrimental effects of a chronic, supra-physiological up-regulation of this pathway, which will likely overshadow the insulinotropic effects of SENP1.

5.2.c.iii Implications for SUMO1 as a therapeutic target.

The present study identifies SUMOylation as a novel regulator of β-cell viability and establishes SUMO1 was an anti-apoptotic protein conferring protection against IL-1β-induced death in INS 832/13 and human islet cells (Figure 23). Conversely, chronic up-regulation of the deSUMOylating enzyme SENP1 reduces insulin secretion secondary to increased apoptotic signaling and reduced viability (Figure 21). Findings that SENP1 knockdown protects against IL-1β-induced death (Figure 22) suggest that manipulation of the SUMOylation pathway may preserve islet mass in type 2 diabetes. This is in apparent

contradiction, however, to the indispensable role of SENP1 in glucose-mediated insulin secretion (Ferdaoussi et al., 2015) and to the proposed role of SENP1 as a regulator of the insulinotropic effects of IL-1β (Figure 33). For the SUMOylation pathway to be recommended as a therapeutic target for the preservation of β-cell mass in vivo, elucidation of the spatial and temporal mechanisms that control the positive and negative roles of SUMOylation in β-cell survival and insulin secretion are required.

While the necessity of deSUMOylation in glucose-stimulated insulin secretion precludes the use of SUMO1 as a therapeutic target in vivo, the ability of SUMO1 conjugation to inhibit stimulus-induced apoptosis in both insulin secreting and human islet cells renders it a promising target for the inhibition of isolation-induced islet loss—a process associated with in vitro β-cell death. To circumvent the use of genetic approaches of intracellular protein modification and to render the findings of this proof-of-concept study clinically translatable, a biologically-active, cell-permeable recombinant TAT-SUMO1 peptide was engineered and purified (Figure 26, Figure 27). Results described in the current studies demonstrate the ability of TAT-SUMO1 to reduce pro-apoptotic gene transcription in IL-1β-treated insulin secreting cells (Figure 28). Furthermore, preliminary evidence indicates that the transpressive actions of TAT-SUMO1 on inflammation- and hypoxia-associated gene expression persists in freshly isolated human islets (Figure 29). Whether the actions of TAT-SUMO1 confer protection against isolation-induced cell death and whether it is sufficient to enhance post-isolation islet mass is currently under investigation.

5.3 FUTURE DIRECTIONS

In an attempt to further understand the pleiotropic effects of IL-1β on glucose homeostasis, the present thesis examined the consequences and regulation of IL-1 signaling both in vivo and in vitro. Findings described here elucidate a positive role for IL-1 signaling in the regulation of glucose homeostasis and identify a novel regulator of IL-1β action in the pancreatic β-cell. Although these findings provide further insight into the pro- and anti-diabetic actions of IL-1β, several questions arising from the present studies remain unanswered:

- What is the relative contribution of the insulinotropic effects of IL-1β in the maintenance of euglycemia following acute metabolic stress?

- Do the insulinotropic actions of IL-1β contribute to the functional decline following exposure to chronic metabolic stress or in T2D?

- What are the molecular mechanisms mediating the acute insulinotropic effects of IL-1β? Are these distinct from those regulating the chronic cytotoxic effects? Are islets of obese non-diabetic donors protected against the detrimental effects of IL-1β?

- What are the mechanisms underlying enhanced sensitivity to the insulinotropic actions of IL-1β in islets of obese donors? How do these mechanisms differ in islets from T2D donors?

- How does SUMOylation modulate IL-1β toxicity? Does SUMOylation also regulate the insulinotropic actions of IL-1β? Are these processes altered in islets of obese or T2D donors?

- Do preliminary findings demonstrating an inhibition of pro-apoptotic signaling by TAT-SUMO1 correlate to reduced post-isolation islet cell death? Does pre-treatment with TAT-SUMO1 improve islet graft survival?

5.4 CONCLUSIONS

Evidence presented in the current thesis demonstrates duration-dependent consequences of IL-1 signaling on glucose homeostasis and β-cell secretory function. In vitro, *acute* IL-1 signaling potentiates glucose-stimulated insulin secretion, which is superseded by induction of cell death subsequent to *persistent* IL-1 stimulation. In vivo, *acute* IL-1 signaling maintains euglycemia by enhancing β-cell secretory response following metabolic stress, whereas *persistent* IL-1 signaling emerges as a regulator of insulin sensitivity. Furthermore, evidence described here suggests that the consequences of IL-1 signaling on glucose homeostasis may also be concentration-dependent, as evidenced by the distinctive glycemic effects induced by acute inflammatory versus acute metabolic stress. Finally, context-dependent consequences of IL-1 signaling are described, with obesogenic environments enhancing IL-1 signaling both in vivo and in vitro, consistent with the context-dependent regulation of IL-1 signaling in inflammatory milieus.

Adding further complexity to the role of IL-1 signaling in glucose homeostasis is understanding its role in T2D. Given the significant genetic and phenotypic heterogeneity of T2D patients and the plethora of factors modulating IL-1 action and activity, IL-1 signaling is unlikely to yield identical glycemic effects in all diabetic patients. Indeed, significant variability in the responses of T2D patients to the anti-diabetic actions of IL-1R antagonism have been reported (Larsen et al., 2007; 2009). It is evident that in certain patients, inhibition of IL-1 signaling ameliorates glycemia. Elucidating the mechanisms regulating the pleiotropic effects of this cytokine will be crucial in identifying the population of diabetic patients in whom inhibition of IL-1 signaling would exacerbate hyperglycemia and identifying those who would benefit most from this therapeutic intervention.

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