Insulin Signaling in the Pancreatic β-Cell

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insulin resistance, insulin receptor, insulin secretion, apoptosis, proliferation, gene expression, diabetes mellitus

Abstract

The appropriate function of insulin-producing pancreatic β-cells is crucial for the regulation of glucose homeostasis, and its impairment leads to diabetes mellitus, the most common metabolic disorder in man. In addition to glucose, the major nutrient factor, inputs from the nervous system, humoral components, and cell-cell communication within the islet of Langerhans act together to guarantee the release of appropriate amounts of insulin in response to changes in blood glucose levels. Data obtained within the past decade in several laboratories have revitalized controversy over the autocrine feedback action of secreted insulin on β-cell function. Although insulin historically has been suggested to exert a negative effect on β-cells, recent data provide evidence for a positive role of insulin in transcription, translation, ion flux, insulin secretion, proliferation, and β-cell survival. Current insights on the role of insulin on pancreatic β-cell function are discussed.

Contents

INTRODUCTION

The hallmark of diabetes mellitus is a disturbance in glucose homeostasis resulting in increased blood glucose levels. Diabetes mellitus has now reached epidemic proportions worldwide and is one of the worst global threats to mankind. Insulin, a 51-amino-acid peptide, is the key hormone responsible for maintaining glucose homeostasis. Absolute deficiency in insulin, as in type 1 diabetes mellitus (T1DM), is due to autoimmune destruction of insulin-producing pancreatic β-cells. Relative deficiency in insulin is due to peripheral insulin resistance and β-cell secretory defect, which is the characteristic of type 2 diabetes mellitus (T2DM). Insulin keeps blood glucose levels within narrow limits by regulating the uptake of glucose by muscle and fat cells as well as regulating hepatic glucose output. To do so, insulin-producing pancreatic β-cells have to secrete insulin in amounts appropriate to the respective blood glucose concentration, a process often referred to as the stimulus-secretion-coupling. In brief, glucose is taken up by β-cells, via glucose transporters, where it is metabolized in glycolysis and Krebs cycle, resulting in an increase in the cytoplasmic ratio of ATP to ADP. This closes ATP-sensitive potassium channels (K_{ATP} channels) and leads to cell membrane depolarization and subsequent opening of voltage-gated Ca^{2+} channels. The resulting increase in cytoplasmic-free Ca^{2+} concentration ($[Ca^{2+}]_i$) finally triggers insulin secretion. As a consequence of this chain of events, insulin secretion is tightly coupled to changes in the extracellular glucose concentration. In adult mammals, pancreatic β-cells are the only source for insulin.

The insulin-producing β-cells, together with glucagon-producing α-cells, somatostatin-producing δ-cells, pancreatic polypeptide (PP)-producing cells, and ghrelin-producing cells, form the microorgan islets of Langerhans. These islets, scattered within the exocrine pancreas and representing approximately 1%–2% of the pancreatic volume (i.e., approximately one million islets in the human pancreas), are highly vascularized by an extensive endothelial network and innervated by sympathetic, parasympathetic, and sensory nerves. In view of these multicellular connections, it is not surprising that the orchestration of multiple signals of different origin guarantees the appropriate function of the β-cell under both basal and glucose-stimulated conditions. These signals include humoral factors (i.e., hormones, vitamins, nutrients, etc.) and nervous stimulation, as well as factors of intraislet cell-cell communication through cell-cell contacts (e.g., connexins, cadherins, ephrins). Whereas the paracrine effects of glucagon (stimulatory), released by α-cells, and of somatostatin (inhibitory), released by δ-cells, on insulin exocytosis by β-cells are well accepted (for review, see 83), the autocrine effect of secreted insulin on β-cell function has been a matter of debate. Although the idea of an autocrine feedback by insulin is not new and dates back to the 1940s (14), both conceptual disagreement and different results in the various experiments have contributed to the controversy, namely whether or not β-cells can be targets for insulin action. Unlike all other cell types, the pancreatic β-cell is unique in that it continuously secretes insulin: basal secretion under nonstimulatory conditions (e.g., low glucose concentration) and increased secretion

T1DM: type 1 diabetes mellitus, insulin-dependent diabetes

T2DM: type 2 diabetes mellitus, non-insulin-dependent diabetes

K_{ATP} channels: ATP-sensitive potassium channels

$[Ca^{2+}]_i$: cytosolic free Ca^{2+} concentration

under stimulatory conditions (e.g., high glucose concentration). Hence, the major argument that led to the conceptual disagreement, i.e., that β-cells cannot respond to insulin, was that β-cells are constantly exposed to insulin and that the respective signal-transduction pathways therefore must be desensitized. The experimental disagreements, for example with regard to the effect of insulin upon insulin secretion, resulted from the fact that all possible outcomes, like negative feedback, positive feedback, and no effect at all, were reported. Although historically insulin was exclusively discussed as a negative signal, recent data provide evidence for a positive role of insulin in several cellular processes that include the regulation of gene transcription, translation, Ca^{2+} flux, β-cell proliferation, and β-cell survival. One of the major points discussed as a source for the controversial results and conceptual disagreement was the question of whether the observed insulin effect upon β-cell function was caused directly by insulin or rather by factors originating from other cell types/tissues in response to the insulin stimulus. This mainly concerned experiments on living animals and perfused pancreata. With regard to studies on isolated islets, a so-called artificial diffusion effect of exogenously administrated insulin was discussed, an effect that resulted in insulin coming the "wrong way," i.e., first stimulating non-β-cells within the mantle of the islet before reaching β-cells in the core of the islet.

The past decade provided us with a wealth of novel experimental data on this conceptually very interesting and important biological question regarding possible effects of insulin on pancreatic β-cell function. Therefore, the aim of the present review is to summarize both historical and recent data with regard to insulin feedback on β-cell function.

INSULIN SIGNALING ELEMENTS OF THE β-CELL

In order to execute its action and initiate a signaling cascade, insulin first has to bind to cell-surface-standing receptors. Usually these are the insulin receptors A type (IR-A) and B type (IR-B). Since insulin at higher concentrations also activates insulin-like growth factor-1 receptors (IGF-1R) (124) and because pancreatic β-cells are surely exposed to insulin concentrations that are higher than those in the periphery, IGF-1R cannot be excluded as targets for insulin binding in these cells. In addition, IR and IGF-1R are able to form hybrid receptors that also bind insulin (10, 11) so that, at least theoretically, β-cells could have six potential receptor types for insulin binding to initiate signaling, e.g., homoreceptors IR-A, IR-B, and IGF-1R as well as hybrid-receptors IR-A/IR-B, IR-A/IGF-1R, and IR-B/IGF-1R. That β-cells are targets for insulin was shown in the 1980s in conventional radio-ligand binding assays (112) as well as by quantitative electron microscopic autoradiography (81). The presence of IR (33) and IGF-1R (44, 110) in insulin-producing cell lines was also reported. Recent data demonstrate the presence of IR-A and IR-B in β-cell lines of different origin (HIT-T15, INS1, MIN6) as well as in primary mouse and human β-cells (39, 61, 74).

Insulin binding to IR, IGF-1R, or hybrid receptors initiates the activation of the intrinsic tyrosine kinase with subsequent autophosphorylation of these receptors and binding and tyrosine phosphorylation of so-called adapter proteins, such as insulin receptor substrate (IRS) proteins IRS-1 to IRS-6, Shc, Gab-1, $p62^{dok}$, and APS. These adaptor proteins provide an interface between the activated receptors and the downstream-located effector molecules (reviewed in 113). Recent data show the expression of IRS-1–4 in mouse β-cells (56), of IRS-1–2 in human β-cells (74), and of Shc in mouse pancreatic islets (109), and several publications document the expression of these adaptor proteins in β-cell lines. Data gathered over the past ten years, coming from both analytical (reverse-transcription polymerase chain reaction and western blotting) and functional studies (transgenic mice, knockout mice, expression of interfering protein variants, and RNAi-mediated knockdown), demonstrated the presence as well as

IR-A: insulin receptor A type, exon 11−

IR-B: insulin receptor B type; exon 11+

IGF-1R: insulin-like growth factor 1 receptor

IRS: insulin receptor substrate

PI3 kinase: phosphoinositide 3-kinase

PKB: protein kinase B, also called Akt

MAP kinases: mitogen-activated protein kinases

PDX-1: pancreatic-duodenal homeobox factor-1, also called insulin-promoter factor-1 (IPF-1)

function of various downstream-located effector proteins, such as PI3 kinase isoforms, isoforms of protein kinase B (PKB, also called Akt), p70s6 kinase, PHAS-1, MAP kinases, and PLCγ (see **Table 1**). However, it was a major breakthrough when Rothenberg et al. (91) and Velloso et al. (111) in 1995 reported that insulin, secreted upon glucose stimulation, activated the β-cell IR and the downstream-located IRS and PI3 kinase. Today we know that insulin activates both the mitogenic (via MAP kinases Erk1/2) and metabolic branches of insulin signaling, the latter involving PI3 kinase, PKB/Akt, mTORC1, and p70s6 kinase, as well as PLCγ. All these studies provided evidence for an autocrine feedback of insulin at the molecular level but did not yet resolve whether insulin is a negative, a positive, or a complex (negative and positive) signal in β-cell function.

INSULIN SIGNALING AND β-CELL FUNCTION

Data from several laboratories, utilizing different experimental approaches, provide new information on the importance of insulin signaling in β-cell physiology/pathology. The approaches range from the generation of conditional knockouts of signaling components to study the phenotype, via in vitro analysis of isolated pancreatic islets or insulin-producing cell lines using pharmacological tools, overexpression of wild-type, or interfering versions of signaling compounds, to RNAi-mediated knockdown of these signaling elements. We focus here on reported effects of insulin signaling on β-cell mass (including β-cell proliferation, β-cell size, and β-cell death), insulin biosynthesis/insulin content, and insulin secretion/Ca^{2+} handling [**Table 2**; **Figure 1**]. We also discuss the concept of β-cell insulin resistance.

β-CELL MASS

The maintenance of pancreatic β-cell mass is achieved by the dynamic balance of neogenesis, proliferation, cell size, and cell death (15). The turnover of β-cell mass is slow under normal conditions, with a proliferation rate and apoptosis rate of approximately 0.5%. However, the β-cell mass can drastically change. For example, during pregnancy β-cell mass is increasing. An increase in β-cell mass is also occurring in the compensatory early phase in T2DM. In the late phase of T2DM, β-cell mass is decreasing due to an enhanced β-cell apoptosis (15).

β-Cell Proliferation and β-Cell Size

Historically, insulin was seen as a negative regulator of β-cell mass/insulin content. In 1941, Best & Haist (14) reported that daily injection of rats with insulin led to a reduced pancreatic insulin content and/or β-cell mass. A genetically engineered knockout of insulin expression led to β-cell hyperplasia in the prenatal state (26), thus supporting this concept. Surprisingly, neither the knockout of the IR in the β-cell in βIRKO mice (53) and the β-cell IGF-1R (54, 123), nor the combined knockout of IR and IGF-1R in βDKO mice (106) led to a change in β-cell mass prenatally. Interestingly, in contrast to the expectation, βIRKO mice show an age-dependent decrease in β-cell mass. In combination with the phenotypes of the β-cell-IGF-$1R^{-/-}$ and the βDKO mice, these data indicate a different function for IR and IGF-1R in the postnatal development of β-cell mass, with a positive impact by the IR-mediated signaling system. Additional and independent information on insulin signaling being a positive regulator of β-cell mass came from studies on IRS-2. The global knockout of IRS-2 leads to the development of a T2DM-like phenotype due to reduced β-cell mass (51, 115). The ablation of IRS-2-dependent signaling in β-cells and hypothalamus by RIP-Cre-mediated knockout (20, 50, 67) as well as a pancreas-restricted knockout, using the pancreatic-duodenal homeobox factor-1 (PDX-1)-promoter-driven Cre system (19), all lead to a similar reduction in β-cell mass with age with no alteration in β-cell mass during early development. Interestingly, although Kubota et al. (50) reported a reduced proliferative activity in 8-week-old mice, no change in

Table 1 Insulin-signaling elements of the pancreatic β-cell

Signaling element	Cell or tissue type	Detection level	Functional characterization	Reference
Receptor				
IR	Rat islets	Radio-ligand binding	no	(81, 112)
IR	RINm5F	WB, IP, radio-ligand binding	yes	(33)
IR	Rat islets	WB, IP	yes	(111)
IR	βTC3	WB	yes	(91)
IR	Rat β-cells	RT-PCR	no	(35)
IR	Mouse β-cells	βIRKO mouse, β-cell KO	yes	(54, 80)
IR	MIN6	Knockdown	yes	(23, 77, 78)
IR-A	HIT, MIN6, INS1,			
	Mouse, rat islets	RT-PCR	yes	(39, 61, 108)
	Human β-cells	Single-cell RT-PCR	yes	(74)
IR-B	HIT, MIN6, INS1,			
	Mouse, rat islets	RT-PCR	yes	(39, 61, 108, 109)
	Human β-cells	Single-cell RT-PCR	yes	(74)
IGF-1R	Rat islets	Radio-ligand binding	no	(110)
	βTC3, HIT-T15	RIA, immunoblotting	no	(44)
	MIN6	Knockdown	yes	(23)
	Mouse β-cells	β-cell KO	yes	(54, 123)
IR/IGF-1R	Mouse β-cells	Double KO	yes	(106)
Adaptor/docking proteins				
IRS-1, -2, -3, -4	Mouse islet, INS1	RT-PCR, IP	yes	(56, 104)
IRS-1	Rat β-cells	Immunohistochemistry	no	(35)
IRS-1	βTC3	WB	yes	(91)
IRS-1, -2	Rat islets	WB, IP	yes	(111)
IRS-1, -2	Human β-cells	Single-cell RT-PCR	no	(74)
IRS-2	Mouse β-cells	β-cell KO	yes	(20, 50, 67)
Gab-1	INS1	IP, enzyme activity	yes	(104)
Shc	Mouse islets, MIN6			
	INS1, HIT-T15, RINm5F	WB, RT-PCR cloning	yes	(109)
Effector proteins				
PI3 kinase Ia	βTC3	Co-IP with IRS-1	no	(91)
p85	MIN6, INS1	siRNA knockdown	yes	(109)
p110α, p110β	Human β-cells	Single-cell RT-PCR	no	(74)
PI3KC2α, PI3KC2γ	Human β-cells	Single-cell RT-PCR	no	(74)
PDK-1	Human β-cells	Single-cell RT-PCR	no	(74)
	HIT-T15	Overexpression, antisense	yes	(61)
	Mouse β-cells	β-cell KO	yes	(37)
PKBα,β,γ (Akt1,2,3)	Human β-cells	Single-cell RT-PCR	no	(74)
PKB	HIT-T15	Enzyme activity		
		overexpression	yes	(61)
PKBα	Mouse β-cells	Transgenic mouse		
		CA-PKB, DN-PKB	yes	(12, 30)

(*Continued*)

Table 1 (*Continued*)

Signaling element	Cell or tissue type	Detection level	Functional characterization	Reference
p70s6k	HIT-T15	Activity, CA-p70s6k	yes	(61, 65)
PHAS-1	βTC6-F7	Phosphorylation	yes	(119)
Erk1/Erk2	INS1	Enzyme activity	yes	(6, 32)
Erk1/2, p70s6k, PKBα,				
GSK3β, PLCγ1	HIT-T15	Enzyme activity	no	(107)

Abbreviations: CA, constitutively active; DN, dominant-negative; IP, immunoprecipitation; KO, knockout; RT-PCR, reverse-transcription polymerase chain reaction; PK, protein kinase; RIA, radioimmunoassay; siRNA, small-interfering RNA; WB, western blotting.

proliferation in β-cells was seen in the study by Lin et al. (67). Another set of data indicating a positive effect of the insulin signaling system in β-cell mass regulation came from experiments on another central player in insulin signal-transduction, namely PKB/Akt (12, 13, 25, 30, 104, 105). Expression of a constitutively active form of PKBα in β-cells of transgenic mice led to an increase in β-cell mass in two parallel reports (13, 105). However, although Bernal-Mizrachi et al. (13) reported an increase in β-cell mass due to both increased proliferation of β-cells (hyperplasia) and increase in β-cell size (hypertrophia), according to Tuttle et al. (105) the observed increase in β-cell mass is solely due to an increase in β-cell size, and in fact, the number of β-cells per islet seems to be reduced. In a complementary study, Bernal-Mizrachi et al. (12) expressed a kinase-dead version of PKBα in β-cells of transgenic mice, which led to an 80% reduction in PKB activity. Interestingly, although these mice showed impaired insulin secretion due to defects at the level of exocytosis, the islet morphology in general and the β-cell mass (β-cell number and size) in particular were not different from those of wild-type controls. Ablation of phosphoinositide-dependent kinase-1 (PDK1), a kinase that activates PKB in response to insulin action, in β-cells resulted in a loss of β-cell mass and led to the development of a T2DM-like phenotype (37). Reduction of β-cell mass was due to both reduced β-cell proliferation and reduced β-cell size. Most interestingly, when ablation of β-cell PDK1 expression was combined with haploinsufficiency of transcription factor Foxo1, which is a target of PKB phosphorylation, the result was a marked increase in β-cell number but not in β-cell size. This result suggests that different mechanisms are involved in the regulation of the hyperplastic versus hypertrophic β-cell phenotype. It remains to be determined whether Foxo1 is involved in the PKB/cyclin-dependent kinase 4–mediated activation of β-cell proliferation (30).

PDK1: phosphoinositide-dependent kinase-1

β-Cell Apoptosis

A further factor to take into account when discussing the regulation of pancreatic β-cell mass is the process of β-cell apoptosis/survival. Although the antiapoptotic action of the insulin/PKB axis is well documented for various tissues and cell types (1), the potential role of the IR/IGF-1R signaling system in pancreatic β-cell protection from apoptosis has been suggested only within the past decade. First lines of evidence for a protective role of insulin came from the global knockout of IRS-2 (115) as well as from the conditional knockout of IR (53), which led to a decrease in β-cell mass. Reduction in β-cell mass in the former model could be prevented by β-cell targeted back expression of IRS-2 (38). Interestingly, conditional knockouts of IRS-2, using either the RIP-Cre (50, 67) or the PDX-1-Cre (19) systems, did not seem to lead to an increase in β-cell apoptosis. The phenotype of mice carrying one or two alleles of the IR while having no IGF-1R expressed in β-cells, i.e., $\beta IR^{+/-}/\beta IGF\text{-}1R^{-/-}$ and $\beta IR^{+/+}/\beta IGF\text{-}1R^{-/-}$, suggested that insulin signaling but not IGF-I signaling is crucial

Table 2 Effect of insulin on β-cell function

Biological function	Effect	Cell or tissue type	Signaling	Reference
Proliferation	Negative	Insulin KO mouse	?	(26)
	Positive	βIRKO mouse, βDKO	IR (postnatal)	(53, 79, 106)
	No effect	βIGFIRKO mouse		(54)
	Positive	Global IRS-2-KO	IRS-2	(51, 115)
	Positive	RIP-Cre/IRS-2-KO	IRS-2	(20, 50)
	No effect	RIP-Cre/IRS-2-KO		(67)
	Positive	PDX-Cre/IRS-2-KO	IRS-2	(19)
	No effect	Transgenic mouse		
		DN-PKB in β-cells		(12)
	Positive	RIP-Cre/PDK1-KO	PDK1	(37)
Antiapoptotic effect	No effect	Transgenic mouse		
		DN-PKB in β-cells		(12)
	No effect	RIP-Cre/IRS-2-KO and		
		PDX-Cre/IRS-2-KO	IRS-2	(19, 50, 67)
	Positive	Human islets	PDX-1	(42)
	Positive	Various	PKB	(25, 28)
	Positive	Various	PKB	(22, 47, 97, 98, 116)
	Positive	RIP-Cre/PDK1-KO	PDK1	(37)
Insulin content	Negative	Various models	?	(14, 49)
	Positive	βTC6-F7/IR	?	(122)
Insulin biosynthesis				
Transcription	Negative	Rat islets	?	(49, 66, 114)
	No effect	Rat islets		(103)
	Positive	HIT, islets (rat, ob mouse)	IR-A/PI3K-Ia/p70s6k	(61, 63, 65, 108)
	Positive	Human islets	IR-A	(74)
	Positive	Various	PI3K Ia	(24, 70)
Insulin biosynthesis				
Transcription	Positive	MIN6, human islets	PDX1-binding	(118)
	Positive	βTC6-F7/IR	?	(122)
	Positive	Various	Various	(3, 23, 95)
Translation	Positive	Rat islets	?	(64)
	Positive	βTC6-F7/IR	?	(122)
Gene expression				
Gene array	Positive/negative	MIN6	?	(77, 78)
Glucokinase	Positive	HIT, islet (rat, ob mouse)	IR-B/PI3K-II/PDK1/PKB	(61, 108, 109)
	Positive	MIN6	IR	(23)
	Positive	βIRKO islets	IR	(80)
c-fos	Positive	HIT, INS1, mouse islets	IR-B/Shc/MEK1/Erk1–2	(109)
Acetyl-CoA carboxylase	Positive	MIN6	SREBP1c	(2)
PDX-1	Positive	Various	Various	(23)
IAPP	Positive	Rat islets	?	(94)

(*Continued*)

Table 2 (*Continued*)

Biological function	Effect	Cell or tissue type	Signaling	Reference
IA-2	Positive	Rat and human islets	?	(68)
Insulin secretion	Negative	Various models	?	(5, 21, 27, 29, 40, 46, 82, 125)
	No effect	Various models		(43, 71, 92, 100, 126)
	Positive	Mouse islets, IRS-1$^{-/-}$ cells	PI3K/IRS-1, SERCA?	(7, 8, 90)
	Positive	βTC6-F7/IR, rat islets	IRS-1, SERCA	(120, 121)
	Negative	Islets (mouse, human)	PI3K	(46, 82)
	No effect	Rat islets		(126)
	Positive	βIRKO mouse	IR	(53, 80)
	Positive	Various	IRS-1	(51, 56, 72, 86)
	Negative	Rat islets	IRS-1	(4)
	Negative	Rat islets	IRS-2	(4)
	Positive	Global IRS-2-KO	IRS-2	(51)
Insulin secretion	Positive	PDX-Cre/IRS-2-KO	IRS-2	(19)
	Positive	Transgenic mouse		
		DN-PKB in β-cells	PKB	(12)
	Complex	Mouse islets		(41)
Cytosolic-free Ca^{2+}	Increase	Mouse islets, IRS-1$^{-/-}$ cells	PI3K/IRS1, SERCA?	(7, 8, 90)
	Increase	βTC6-F7/IR, rat islets	IRS1, SERCA	(17, 18, 120, 121)
	Decrease	Mouse islets	PI3K	(46)

Abbreviations: CA, constitutively active; DN, dominant negative; IR, insulin receptor; IRS, insulin receptor substrate; KO, knockout; PDX, pancreatic-duodenal homeobox factor; PK, protein kinase; SERCA, sarco/endoplasmic reticulum Ca^{2+}-ATPase; SREBP, sterol regulatory element-binding protein.

for protection against β-cell apoptosis (106). Further evidence came from the observation that the Arg972 polymorphism in the human IRS-1 gene of diabetic and prediabetic carriers causes apoptosis of β-cells (31). Although there is quite compelling evidence for the involvement of PKB in β-cell protection, and several potential mechanisms have been discussed (for review, see 22, 25, 28 and references therein, 47, 97, 98, 116), other studies put the requirement of PKB in question. Whereas Johnson et al. (42) clearly see a β-cell protective role of low doses of insulin (0.2–20 nM) in terms of serum withdrawal–induced apoptosis, these doses do not seem to activate PKB. Moreover, an 80% reduction of PKB activity in transgenic mice expressing a dominant-negative version of PKB in β-cells led to no change in β-cell apoptosis when compared with wild-type mice (12). Although ablation in β-cells of the PKB-activating kinase PDK1 increases their susceptibility to apoptosis (37), it should be noted that PKB is not the only substrate of PDK1 and that the observed effect therefore might be PKB independent.

INSULIN BIOSYNTHESIS

As mentioned above (see β-Cell Proliferation and β-Cell Size), the first note on a potential negative feedback action of insulin on β-cell function was related to insulin biosynthesis/insulin content (14). The negative effect of administrated insulin on insulin biosynthesis was in agreement with other reports on the effect of transplanted insulinomas, which also led to a reduction in insulin content/insulin biosynthesis (see 49). Because chronic administration

of insulin is associated with hypoglycemia, a condition resulting in reduced insulin biosynthesis, Koranyi et al. (49) combined hyperinsulinemic clamps with glucose clamps to circumvent this problem. Employing a 12-h insulin infusion at fixed glucose levels again suggested a negative effect of insulin on insulin biosynthesis but did not reveal whether the insulin effect was a direct one.

Although it has been suggested that insulin reduces insulin biosynthesis, it is now clear that insulin has a positive effect on its own production (64, 65, 122). Although these findings were controversial in the light of reports showing no or a negative effect of insulin on its own biosynthesis (66, 103, 114), further data strongly supported this concept (3, 23, 24, 69, 74, 95, 118).

A matter of confusion was caused by the observation that islets of animals, where components of the β-cell insulin signaling machinery were knocked out, still contained insulin mRNA. However, this is not surprising because basal insulin gene transcription is regulated differently from glucose/insulin-stimulated insulin gene transcription (62, 63).

With regard to the molecular mechanisms involved in insulin-dependent insulin biosynthesis, most of the available data are for the insulin-dependent upregulation of transcription. Because of the potentially very high levels of insulin at the β-cell surface following exocytosis, it was unclear whether insulin activates the transcription by signaling via the IR or through IGF-1R. Although overexpression of IR led to a pronounced effect on insulin-stimulated insulin gene expression (65, 122), neither stimulation with IGF-I nor blocking of IGF-1R affected the upregulation of insulin promoter activity (61, 65), thus suggesting the involvement of IR. The most convincing evidence came from experiments on islets of βIRKO mice, which express IGF-1R but not IR (53). Exposure of these islets to either elevated glucose concentration or exogenous insulin did not result in the upregulation of endogenous insulin gene transcription, as was the case in islets prepared from control animals (61). The involvement of IR rather than IGF-1R in stimulated insulin gene transcription was independently confirmed by an RNAi-mediated knockdown approach for each receptor (23). More detailed experiments revealed that insulin activates its own gene by signaling via IR-A (61, 74), here initiating the signaling cascade from plasma membrane-standing receptors (108) with further signaling through IRS (56, 65), class Ia PI3K (24, 61, 65, 70), and p70s6k (65). In agreement with PDX-1 being one of the transcription factors involved in glucose-stimulated upregulation of insulin gene transcription, Wu et al. reported increased binding of PDX-1 to its binding sites in the insulin promoter in response to insulin (118).

Reports from several laboratories have documented the transcriptional regulation of additional genes by insulin, such as the glucokinase gene (23, 61, 80), i.e., the so-called glucose-sensor of the β-cell. Moreover, gene expression profiling of MIN6 cells revealed that most genes that seemed to be regulated by glucose were in fact regulated by secreted insulin (77, 78). This might be explained, at least in part, by the observation that the expression as well action of several important transcription factors of the β-cell, e.g., PDX-1, Foxo1, and Foxa2, are regulated by insulin (23, 45, 48, 57, 73, 117, 118).

INSULIN SECRETION

The most controversial topic with regard to insulin feedback and β-cell function is the role of insulin in insulin secretion. Published data have led to four possible outcomes, i.e., that insulin is (*a*) a negative regulator, (*b*) a positive regulator, (*c*) essential, or (*d*) not involved. Historically, insulin exocytosis was suggested to be inhibited by secreted insulin (27, 40), and several publications support this view (5, 21, 29, 46, 82, 125, 126). Although other historical and recent reports demonstrate no effects of insulin on its own secretion (43, 71, 92, 100, 126), another recent line of evidence suggests that secreted insulin may be essential for insulin exocytosis or even have a positive effect on its

SERCA: sarco/endoplasmic reticulum Ca^{2+}-ATPase

own release (7, 8, 18, 53, 55, 56, 80, 90, 99, 120, 121).

Aspinwall et al. (7) were the first to report that secreted insulin stimulates the immediate, ongoing process of insulin exocytosis. A second line of evidence came from the growing number of general and conditional knockout models for proteins involved in insulin signal transduction. Several of these animal models showed impaired glucose tolerance due to impaired insulin release. Islets from mice with a global knockout of IRS-1 (51, 56), or with a β-cell knockout of IR (53, 80, 106) and IGF-1R (54, 106, 123), or with an islet cell knockout of IRS-2 (19), exhibit a marked insulin secretory defect in response to glucose. Finally, a third line of evidence came from reports on diabetic and prediabetic carriers of the Arg972 polymorphism in the IRS-1 gene, which is linked with a defect in insulin secretion (72, 86).

Although the majority of data allow the conclusion that β-cell insulin signaling is essential for insulin exocytosis, it remains unclear what role different components of the insulin-signaling cascade(s) play. For example, disruption of the same targets by different approaches led to controversial results. Experiments by Kubota et al. (51) with islets of IRS-$1^{-/-}$ mice demonstrated a reduced secretory response to glucose, whereas islets of IRS-$2^{-/-}$ mice showed an increase in glucose-stimulated insulin release. The opposite outcome was reported by Araujo et al. (4), who used an antisense-DNA-oligonucleotide knockdown approach. Here, knockdown of IRS-1 in rat islets led to an increase in glucose-induced insulin release, whereas knockdown of IRS-2 had no effect. Cantley et al. (19) used islets from a PDX-1-Cre-driven islet-specific IRS-2 knockout and showed a decrease in glucose-stimulated insulin secretion. This means that care must be taken when linking the long-term knockout and knockdown effect(s) in knockout animals and stable knockdown cell lines with the acute regulation demonstrated in the biochemical experiments discussed below.

Some reports have suggested that positive feedback on exocytosis via the IR/IGF-1R system is due to insulin-mediated increases in $[Ca^{2+}]_i$ (7, 8, 17, 18, 55, 90, 120, 121). Aspinwall et al. (7, 8) demonstrate an insulin-stimulated increase in $[Ca^{2+}]_i$ within seconds after start of stimulation, which originates from intracellular Ca^{2+} stores rather than from extracellular Ca^{2+} entry and involves IRS-1 and PI3 kinase activity. Similar dynamics with regard to increases in $[Ca^{2+}]_i$ and insulin release were observed by Roper et al. (90) when using the fungal insulin mimetic L-783,281, which again suggests that signal transduction occurs via IRS-1 and PI3 kinase. Experiments from Wolf's laboratory (120, 121) also identify IRS-1 as a regulator of insulin-dependent changes in $[Ca^{2+}]_i$ but suggest a different mechanism. Although some data suggest a dependency on PI3 kinase and Ca^{2+} mobilization from the endoplasmic reticulum (7, 8, 90), results by Wolf et al. (120, 121) demonstrate that insulin-mediated changes in $[Ca^{2+}]_i$ are PI3 kinase independent and that $[Ca^{2+}]_i$ increases because of an IRS-1-dependent blockade of the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA). These controversies, however, may be explained by the different experimental setups. In one study (7, 8), only a 30-second insulin stimulus was used and thus the data reflect the immediate effect of insulin signaling, whereas the data described in another study (120, 121) were obtained after a 72-hour exposure to elevated insulin and therefore may be the result of later, second wave responses that are PI3 kinase independent. In fact, both observations may be true and may reflect the situation at the time point studied.

Insulin could affect changes in $[Ca^{2+}]_i$ through the regulation of glucose sensing/metabolism and the resulting change in ATP/ADP ratio, thereby affecting membrane potential as well as the action of Ca^{2+} pumps. Data from β-cell IR/IGF-1R double-knockout islets show impairments in glucose-dependent O_2 consumption (106). Although expression of the β-cell glucose-sensor glucokinase (23, 61, 80, 109) and recruitment of active glucokinase molecules from the inactive pool (88, 89) were shown to be insulin-dependent, and insulin has

been implicated in the autocrine regulation of β-cell glucose metabolism (16), the dependence of glucose metabolism on insulin feedback has yet to be further elucidated.

In addition to elevating $[Ca^{2+}]_i$, insulin signaling also may directly affect the exocytotic machinery. Transgenic mice expressing a dominant-negative version of PKB in their β-cells show a defect in basal as well as in glucose-stimulated or KCl-stimulated insulin release. Because the islets of these animals exhibit normal Ca^{2+} responses toward stimulation with either glucose or KCl, this suggests the involvement of PKB activity in the maintenance of the distal exocytotic machinery (12).

However, the findings discussed above do not settle the old dispute on whether the insulin effect on exocytosis is positive or negative. In addition to the reports of positive effects of insulin, new arguments support an immediate negative feedback (46, 82). These latter studies show that the K_{ATP} channel is a potential target for insulin to regulate its own secretion. Experiments in neurons have suggested that stimulation by insulin via the activation of PI3 kinase leads to an increase in PI(3,4,5)P_3, which opens the K_{ATP} channel (96). If this were to occur in the β-cell, the glucose-stimulus/insulin-secretion coupling should be switched off and insulin secretion would be stopped. In parallel work on the rat β-cell line GRI-G1, the same group was unable to see similar effects on the K_{ATP} channel, although PI(3,4,5)P_3 levels were significantly increased (36). However, recent studies on normal mouse and human β-cells have provided evidence that K_{ATP} channels can be opened by insulin via PI3 kinase (46, 82), most likely due to PI(3,4,5)P_3 (9, 58), which leads to an inactivation of insulin secretion caused by hyperpolarization of the plasma membrane. Most interestingly, recent data by Hagren & Tengholm (34) show glucose/insulin-induced oscillations of PI(3,4,5)P_3 in MIN6 cells. It remains to be clarified whether these oscillations are the consequence of oscillatory insulin release (85), which may be driven by the oscillations in the ATP/ADP ratio and $[Ca^{2+}]_i$ (59, 76), or whether these oscillations contribute to pulsatile insulin release by rapidly opening and closing K_{ATP} channels. Eto et al. (29) report that inhibition of PI3 kinase did not interfere with glucose-oxidation, ATP content, or $[Ca^{2+}]_i$, but suggest the effect to be distal to the increase in $[Ca^{2+}]_i$.

Finally, although some recent reports provide evidence that insulin has neither a positive nor a negative effect on insulin secretion (43, 69, 126), data by Jimenez-Feltstrom et al. (41) suggest that the effect of insulin is dose dependent. Although low insulin concentrations, i.e., from 0.05 to 0.1 nM, stimulate insulin release from mouse islets, concentrations between 1 and 100 nM have no effect on insulin exocytosis, and concentrations higher than 250 nM finally inhibit insulin exocytosis in a PI3 kinase/NO synthase–dependent manner. These data support the view that insulin may be a rather complex signal in the regulation of its own secretion. Whereas basal insulin may serve as a maintenance signal that primes the β-cell to respond to the next glucose stimulus, insulin may inhibit further release at the peak of the exocytotic event, i.e., at very high local insulin concentration. For the latter to occur, however, would require that IR/IGF-1R are located in close proximity to the exocytotic events, which remains to be shown.

β-CELL INSULIN RESISTANCE

The data discussed above clearly demonstrate that the insulin-producing pancreatic β-cell is a target for insulin action, with insulin effects on transcription, translation, glucose and lipid metabolism, ion flux, cell proliferation, cell size, and β-cell apoptosis. As a consequence, mechanisms leading to insulin resistance in the classical insulin-target tissues, namely liver, muscle and fat, should also affect β-cell function and survival, i.e., insulin resistance is also a feature of the pancreatic β-cell.

Although T2DM was previously considered to be caused either by peripheral insulin resistance or by pancreatic β-cell dysfunction (reviewed in 87, 102), it is now clear that it is caused by the combination of both. The pancreatic

β-cell becomes unable to respond to the increased demands, i.e., secreting more and more insulin to overcome the prevailing insulin resistance. The current view with regard to insulin resistance is that in addition to liver, muscle, fat, and brain, the β-cell also is affected. In the early state of the disease, β-cells still secrete sufficient amounts of insulin, mainly due to hypersecretion and/or compensatory increase of β-cell mass by hyperplasia, to compensate for insulin resistance and to regulate hepatic glucose output. However, what is never spelled out is that it is not a normal, healthy β-cell that has to cope with the higher demand for insulin. An increasing body of data documents that mechanisms that lead to insulin resistance in the classical insulin-target tissues also cause β-cell insulin resistance coupled with β-cell dysfunction and apoptosis (3, 22, 72, 79, 93, 95). These data suggest that β-cell insulin resistance may add to the deterioration of β-cell function and therefore accelerate the progression of the disease.

CONCLUSION

The wealth of data, both historical and those gathered over the past 10 years, provide strong evidence that the pancreatic β-cell indeed is a target for insulin action. However, additional work is needed to solve the still existing controversies, especially with regard to insulin's action upon its secretory process. As contradictory as these reports might look at first glance, perhaps the different experimental approaches/conditions employed may offer an explanation to the different outcomes, as explained below.

One critical aspect is the dynamics of events that are triggered by insulin. Secreted insulin activates its own receptor and PI3 kinase within seconds. This forms the basis for an immediate, i.e., within seconds to a few minutes, influence on cellular events. The next level of insulin action regulates processes within several minutes, such as phosphorylation/dephosphorylation, protein translocation, protein-protein interaction, and protein-DNA interaction, but does not require the synthesis of new proteins. These activities may regulate processes such as enzyme activity, ion channel activity, gene transcription, and translation. Longer-term effects of insulin feedback action, i.e., after 30 minutes, may be achieved by the synthesis of proteins, including transcription factors that regulate expression of additional genes. Moreover, insulin action may trigger the sequential activation of positive/activating and negative/inactivating signals. This may involve the sequential activation of tyrosine kinases/phosphatases and serine/threonine kinases/phosphatases, similar to the sequence of events that lead to the activation (i.e., tyrosine phosphorylation by the IR) and later inactivation (i.e., serine phosphorylation by insulin-activated atypical PKC) of IR and IRS (127). Consequently, the time point at which the insulin effect on a certain β-cell function is studied will have an impact on the result and, hence, interpretation.

Another explanation for the controversy in results may come from the mode in which β-cells were exposed to insulin. In physiology, i.e., under basal as well as stimulated conditions, pancreatic β-cells secrete insulin in a pulsatile manner (85). The physiological significance of this oscillatory insulin release has been discussed in the context of it being a more effective signal for peripheral target tissues. However, this pulsatile insulin release may be even more important for the β-cell itself since it guarantees that pancreatic β-cells can respond to insulin at all. Continuous exposure of β-cells to high levels of insulin, as is the case in insulin-clamp studies or as a result of long-term incubation of β-cells with static insulin or insulin secretagogues, may indeed result in a negative feedback, or even in the lack of response, resulting from the desensitization of the signaling cascade. It is noteworthy that loss of pulsatile insulin release is observed already in the early stages of T2DM (85).

With regard to different outcomes when using gene knockout mouse models, it became evident that the genetic background can influence the phenotype. Kulkarni et al. (52) studied the

influence of genetic background in three mouse strains, i.e., C57BL/B6, 129Sv, and DBA, that carried a double-heterozygous knockout for the IR and IRS-1. Whereas mice on a B6 background exhibited marked hyperinsulinemia and islet hyperplasia and were overtly diabetic by six months of age, mice on a 129Sv background showed a mild hyperinsulinemia and minimal hyperplasia, and only 2% of the mice became diabetic. Mice on a DBA background developed an intermediate phenotype. Suzuki et al. (101) reported a similar dependency on the genetic background for PDX-1 expression levels in IRS-$2^{-/-}$ mice. A further complication for the interpretation of results was identified for β-cell conditional knockout models based on the RIP-Cre [B6.Cg-Tg(Ins2-cre)25Mgn/J] system: The expression of the Cre-recombinase is driven by a –690 bp fragment of the rat insulin-2 promoter. Although it was initially believed that in these mice the Cre recombinase is selectively expressed in pancreatic β-cells, thus leading to a β-cell-specific conditional knockout of floxed candidate genes, it has recently been shown that the Cre-recombinase is also expressed, to different degrees, in regions of the brain including the hypothalamus. This might result in the development of obesity and hyperleptinemia, which per se might affect β-cell function (20, 50, 67). Moreover, it has been suggested that RIP-Cre mice themselves develop a mild glucose intolerance, perhaps as a consequence of the genetic background of the mouse strain (60, 84).

Taken together, a growing body of evidence demonstrates that autocrine insulin feedback, resulting in both positive and negative effects, is involved in the regulation of β-cell function. However, it has to be pointed out that under normal, physiological conditions, insulin feedback takes place at a time when the pancreatic β-cells face a plethora of signals that accompany food intake and digestion. These include neural factors, the action of incretines, intraislet cell-cell communication, elevated glucose metabolism and elevated $[Ca^{2+}]_i$ levels, and last but not least the potential feedback action of factors cosecreted with insulin. All these factors, in addition to providing the basis for a complex cross talk between various signaling pathways in pancreatic β-cell function, will also affect insulin-induced signal transduction.

In addition to producing β-cell-specific insights, research activities in β-cell insulin signaling have revitalized more general aspects in insulin signal transduction. Although it has been known since 1985 that the IR exists as two isoforms, i.e., IR-A and IR-B, the biological roles of the two different IR remained unclear. Work on the pancreatic β-cell showed for the first time that the two IR isoforms can indeed signal differently and thus contribute to the selectivity in insulin action. While signaling through IR-A activates the transcription of the insulin gene, simultaneous signaling via IR-B upregulates the expression of the glucokinase and c-fos genes within the same cell (61, 65, 109). Future work will have to disclose different functions of the two IRs in other cell types. The finding that both IR isoforms can be located in different plasma membrane microdomains and activate different signaling cascades (61, 108) was discovered to be true also for non-β-cells (61), which has implications for insulin signal transduction beyond the β-cell. The molecular mechanisms underlying the segregation of IR-A and IR-B into different plasma membrane microdomains remain to be clarified. Detailed analysis of selective signaling via IR-B in β-cells and the activation of c-fos gene transcription lends support to the general concept that IR can also signal from intracellular compartments following their endocytosis, here the early/sorting endosomes (109).

Future research on β-cell insulin signaling will not only have to clarify which processes that were previously described as neural/incretine/paracrine/glucose dependent are in fact insulin dependent, but also will have to show to what extent maintenance of β-cell function is dependent on the autocrine insulin feedback action under basal and stimulated conditions.

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The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

LITERATURE CITED

1. Alessi DR, Downes CP. 1998. The role of PI 3-kinase in insulin action. *Biochim. Biophys. Acta* 1436:151–64
2. Andreolas C, da Silva Xavier G, Diraison F, Zhao C, Varadi A, et al. 2002. Stimulation of acetyl-CoA carboxylase gene expression by glucose requires insulin release and sterol regulatory element binding protein 1c in pancreatic MIN6 β-cells. *Diabetes* 51:2536–45
3. Andreozzi F, D'Alessandris C, Federici M, Laratta E, del Guerra S, et al. 2004. Activation of the hexosamine pathway leads to phosphorylation of insulin receptor substrate-1 on Ser307 and Ser612 and impairs the phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin insulin biosynthetic pathway in RIN pancreatic β-cells. *Endocrinology* 145:2845–57
4. Araujo EP, Amaral MEC, Filiputti E, de Souza CT, Laurito TL, et al. 2004. Restoration of insulin secretion in pancreatic islets of protein-deficient rats by reduced expression of insulin receptor substrate (IRS)-1 and IRS-2. *J. Endocrinol.* 181:25–28
5. Araujo EP, Amaral MEC, Souza CT, Bordin S, Ferreira F, et al. 2002. Blockade of IRS1 in isolated rat pancreatic islets improves glucose-induced insulin secretion. *FEBS Lett.* 531:437–42
6. Arnette D, Gibson TB, Lawrence MC, January B, Shih K, et al. 2003. Regulation of ERK1 and ERK2 by glucose and peptide hormones in pancreatic β cells. *J. Biol. Chem.* 278:32517–25
7. Aspinwall CA, Lakey JRT, Kennedy RT. 1999. Insulin-stimulated insulin secretion in single pancreatic beta cells. *J. Biol. Chem.* 274:6360–65
8. Aspinwall CA, Qian WJ, Roper MG, Kulkarni RN, Kahn CR, Kennedy RT. 2000. Roles of insulin receptor substrate-1, phosphatidylinositol 3-kinase, and release of intracellular Ca^{2+} stores in insulin-stimulated insulin secretion in β-cells. *J. Biol. Chem.* 275:22331–38
9. Barker CJ, Leibiger IB, Leibiger B, Berggren PO. 2002. Phosphorylated inositol compounds in β-cell stimulus-response coupling. *Am. J. Physiol. Endocrinol. Metab.* 283:E1113–22
10. Belfiore A. 2007. The role of insulin receptor isoforms and hybrid insulin/IGF-I receptors in human cancer. *Curr. Pharm. Des.* 13:671–86
11. Benyoucef S, Surinya KH, Hadaschik D, Siddle K. 2007. Characterization of insulin/IGF hybrid receptors: contributions of the insulin receptor L2 and Fn1 domains and the alternatively spliced exon 11 sequence to ligand binding and receptor activation. *Biochem. J.* 403:603–13
12. Bernal-Mizrachi E, Fatrai S, Johnson JD, Ohsugi M, Otani K, et al. 2004. Defective insulin secretion and increased susceptibility to experimental diabetes are induced by reduced Akt activity in pancreatic islet β cells. *J. Clin. Invest.* 114:928–36
13. Bernal-Mizrachi E, Wen W, Stahlhut S, Welling CM, Permutt MA. 2001. Islet β cell expression of constitutively active Akt1/PKBα induces striking hypertrophy, hyperplasia, and hyperinsulinemia. *J. Clin. Invest.* 108:1631–38
14. Best CH, Haist RE. 1941. The effect of insulin administration on the insulin content of the pancreas. *J. Physiol.* 100:142–46
15. Bonner-Weir S. 2000. Life and death of the pancreatic beta cells. *Trends Endocrinol. Metab.* 11:375–78

16. Borelli MI, Francini F, Gagliardino JJ. 2004. Autocrine regulation of glucose metabolism in pancreatic islets. *Am. J. Physiol. Endocrinol. Metab.* 286:E111–15
17. Borge PD, Moibi J, Greene SR, Trucco M, Young RA, et al. 2002. Insulin receptor signaling and sarco/endoplasmic reticulum calcium ATPase in β-cells. *Diabetes* 51:S427–33
18. Borge PD, Wolf BA. 2003. Insulin receptor substrate 1 regulation of sarco-endoplasmic reticulum calcium ATPase 3 in insulin-secreting β-cells. *J. Biol. Chem.* 278:11359–68
19. Cantley J, Choudhury AI, Asare-Anane H, Selman C, Lingard S, et al. 2007. Pancreatic deletion of insulin receptor substrate 2 reduces beta and alpha cell mass and impairs glucose homeostasis in mice. *Diabetologia* 50:1248–56
20. Choudhury AI, Heffron H, Smith MA, Al-Qassab H, Xu AW, et al. 2005. The role of insulin receptor substrate 2 in hypothalamic and β cell function. *J. Clin. Invest.* 115:940–50
21. Collier JJ, White SM, Dick GM, Scott DK. 2004. Phosphatidylinositol 3-kinase inhibitors reveal a unique mechanism of enhancing insulin secretion in 832/13 rat insulinoma cells. *Biochem. Biophys. Res. Commun.* 324:1018–23
22. D'Alessandris C, Andreozzi F, Federici M, Cardellini M, Brunetti A, et al. 2004. Increased O-glycosylation of insulin signaling proteins results in their impaired activation and enhanced susceptibility to apoptosis in pancreatic β-cells. *FASEB J.* 18:959–61
23. da Silva Xavier G, Qian Q, Cullen PJ, Rutter GA. 2004. Distinct roles for insulin and insulin-like growth factor-1 in pancreatic β-cell glucose sensing revealed by RNA silencing. *Biochem. J.* 377:149–58
24. da Silva Xavier G, Varadi A, Ainscow EK, Rutter GA. 2000. Regulation of gene expression by glucose in pancreatic β-cells (MIN6) via insulin secretion and activation of phosphatidylinositol 3'-kinase. *J. Biol. Chem.* 275:36269–77
25. Dickson LM, Rhodes CJ. 2004. Pancreatic β-cell growth and survival in the onset of type 2 diabetes: a role for protein kinase B in the Akt? *Am. J. Physiol. Endocrinol. Metab.* 287:E192–98
26. Duville B, Currie C, Chrones T, Bucchini D, Jami J, et al. 2002. Increased islet cell proliferation, decreased apoptosis, and greater vascularization leading to β-cell hyperplasia in mutant mice lacking insulin. *Endocrinology* 143:1530–37
27. Elahi D, Nagulesparan M, Hershcopf RJ, Muller DC, Tobin JD, et al. 1982. Feedback inhibition of insulin secretion by insulin: relation to the hyperinsulinemia of obesity. *N. Engl. J. Med.* 306:1196–202
28. Elghazi L, Balcazar N, Bernal-Mizrachi E. 2006. Emerging role of protein kinase B/Akt signaling in pancreatic beta-cell mass and function. *Int. J. Biochem. Cell Biol.* 38:157–63
29. Eto K, Yamashita T, Tsubamoto Y, Terauchi Y, Hirose K, et al. 2002. Phosphatidylinositol 3-kinase suppresses glucose-stimulated insulin secretion by affecting postcytosolic [Ca^{2+}] elevation signals. *Diabetes* 51:87–97
30. Fatrai S, Elghazi L, Balcazar N, Cras-Meneur C, Krits I, et al. 2006. Akt induces β-cell proliferation by regulating cyclin D1, cyclin D2, and p21 levels and cyclin-dependent kinase-4 activity. *Diabetes* 55:318–25
31. Federici M, Hribal ML, Ranalli M, Marselli L, Porzio O, et al. 2000. The common Arg972 polymorphism in insulin receptor substrate-1 causes apoptosis of human pancreatic islets. *FASEB J.* 15:22–24
32. Frodin M, Sekine N, Roche E, Filloux C, Prentki M, et al. 2003. Glucose, other secretagogues, and nerve growth factor stimulate mitogen-activated protein kinase in the insulin-secreting β-cell line, INS-1. *J. Biol. Chem.* 270:7882–89
33. Gazzano H, Halban P, Prentki M, Ballotti R, Brandenburg D, et al. 1985. Identification of functional insulin receptors on membranes from an insulin-producing cell line (RINm5F). *Biochem. J.* 226:867–72
34. Hagren OI, Tengholm A. 2006. Glucose and insulin synergistically activate phosphatidylinositol 3-kinase to trigger oscillations of phosphatidylinositol 3,4,5-trisphosphate in beta-cells. *J. Biol. Chem.* 281:39121–27
35. Harbeck MC, Louie DC, Howland J, Wolf BA, Rothenberg PL. 1996. Expression of insulin receptor mRNA and insulin receptor substrate 1 in pancreatic islet beta-cells. *Diabetes* 45:711–17
36. Harvey J, McKenna F, Herson PS, Spanswick D, Ashford ML. 1997. Leptin activates ATP-sensitive potassium channels in the rat insulin-secreting cell line, CRI-G1. *J. Physiol.* 504(Pt. 3):527–35
37. Hashimoto N, Kido Y, Uchida T, Asahara S, Shigeyama Y, et al. 2006. Ablation of PDK1 in pancreatic cells induces diabetes as a result of loss of cell mass. *Nat. Genet.* 38:589–93

38. Hennige AM, Burks DJ, Ozcan U, Kulkarni RN, Ye J, et al. 2003. Upregulation of insulin receptor substrate-2 in pancreatic β cells prevents diabetes. *J. Clin. Invest.* 112:1521–32
39. Hribal ML, Perego L, Lovari S, Andreozzi F, Menghini R, et al. 2003. Chronic hyperglycemia impairs insulin secretion by affecting insulin receptor expression, splicing, and signaling in RIN β cell line and human islets of Langerhans. *FASEB J.* 17:1340–42
40. Iversen J, Miles DW. 1971. Evidence for a feedback inhibition of insulin on insulin secretion in the isolated, perfused canine pancreas. *Diabetes* 20:1–9
41. Jimenez-Feltstrom J, Lundquist I, Obermuller S, Salehi A. 2004. Insulin feedback actions: complex effects involving isoforms of islet nitric oxide synthase. *Regul. Pept.* 122:109–18
42. Johnson JD, Bernal-Mizrachi E, Alejandro EU, Han Z, Kalynyak TB, et al. 2006. Insulin protects islets from apoptosis via Pdx1 and specific changes in the human islet proteome. *Proc. Natl. Acad. Sci. USA* 103:19575–80
43. Johnson JD, Misler S. 2002. Nicotinic acid-adenine dinucleotide phosphate-sensitive calcium stores initiate insulin signaling in human beta cells. *Proc. Natl. Acad. Sci. USA* 99:14566–71
44. Katz LEL, Bhala A, Camron E, Nunn SE, Hintz RL, Cohen P. 1997. IGF-II, IGF-binding proteins and IGF receptors in pancreatic beta-cell lines. *J. Endocrinol.* 152:455–64
45. Kawamori D, Kaneto H, Nakatani Y, Matsuka T, Matsuhisa M, et al. 2006. The forkhead transcription factor FoxO1 bridges the JNK pathway and the transcription factor PDX-1 through its intracellular translocation. *J. Biol. Chem.* 281:1091–98
46. Khan FA, Goforth PB, Zhang M, Satin LS. 2001. Insulin activates ATP-sensitive K^+ channels in pancreatic β-cells through a phosphatidylinositol 3-kinase-dependent pathway. *Diabetes* 50:2192–98
47. Kim S-J, Winter K, Nian C, Tsuneoka M, Koda Y, McIntosh CHS. 2005. Glucose-dependent insulinotropic polypeptide (GIP) stimulation of pancreatic β-cell survival is dependent upon phosphatidylinositol 3-kinase (PI3K)/protein kinase B (PKB) signaling, inactivation of the forkhead transcription factor Foxo1, and downregulation of bax expression. *J. Biol. Chem.* 280:22297–307
48. Kitamura T, Nakae J, Kitamura Y, Kido Y, Bigs WH III, et al. 2002. The forkhead transcription factor *Foxo1* links insulin signaling to *Pdx1* regulation of pancreatic β cell growth. *J. Clin. Invest.* 110:1839–47
49. Koranyi L, James DE, Kraegen EW, Permutt MA. 1992. Feedback inhibition of insulin gene expression by insulin. *J. Clin. Invest.* 89:432–36
50. Kubota N, Terauchi Y, Tobe K, Yano W, Suzuki R, et al. 2004. Insulin receptor substrate 2 plays a crucial role in ß cells and the hypothalamus. *J. Clin. Invest.* 114:917–27
51. Kubota N, Tobe K, Terauchi Y, Eto K, Yamauchi T, et al. 2000. Disruption of insulin receptor substrate 2 causes type 2 diabetes because of liver insulin resistance and lack of compensatory beta-cell hyperplasia. *Diabetes* 49:1880–89
52. Kulkarni RN, Almind K, Goren HJ, Winnay JN, Ueki K, et al. 2003. Impact of genetic background on development of hyperinsulinemia and diabetes in insulin receptor/insulin receptor substrate-1 double heterozygous mice. *Diabetes* 52:1528–34
53. Kulkarni RN, Bruning JC, Winnay JN, Postic C, Magnuson MA, Kahn CR. 1999. Tissue-specific knockout of the insulin receptor in pancreatic β cells creates an insulin secretory defect similar to that in type 2 diabetes. *Cell* 96:329–39
54. Kulkarni RN, Holzenberger M, Shih DQ, Ozcan U, Stoffel M, et al. 2002. β-cell specific deletion of the IGF1 receptor leads to hyperinsulinemia and glucose intolerance but does not alter β-cell mass. *Nat. Genet.* 31:111–15
55. Kulkarni RN, Roper MG, Dahlgren G, Shih DQ, Kauri LM, et al. 2004. Islet secretory defects in insulin receptor substrate 1 null mice is linked with reduced calcium signaling and expression of sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA)-2b and -3. *Diabetes* 53:1517–25
56. Kulkarni RN, Winnay JN, Daniels M, Bruning JC, Flier SN, et al. 1999. Altered function of insulin receptor substrate-1-deficient mouse islets and cultured β-cell lines. *J. Clin. Invest.* 104:R69–75
57. Kushner JA, Ye J, Schubert M, Burks DJ, Dow MA, et al. 2002. Pdx1 restores β cell function in Irs2 knockout mice. *J. Clin. Invest.* 109:1193–201
58. Larsson O, Barker CJ, Berggren PO. 2000. Phosphatidylinositol 4,5-bisphosphate and ATP-sensitive potassium channel regulation. A word of caution. *Diabetes* 49:1409–12

59. Larsson O, Kindmark H, Brandstrom R, Fredholm B, Berggren PO. 1996. Oscillations in KATP channel activity promote oscillations in cytoplasmic free Ca^{2+} concentration in the pancreatic beta cell. *Proc. Natl. Acad. Sci. USA* 93:5161–65
60. Lee JY, Ristow M, Lin X, White MF, Magnuson MA, Hennighausen L. 2006. RIP-Cre revisited, evidence for impairments of pancreatic β-cell function. *J. Biol. Chem.* 281:2649–53
61. Leibiger B, Leibiger IB, Moede T, Kemper S, Kulkarni RN, et al. 2001. Selective signaling through A and B insulin receptors regulates transcription of insulin and glucokinase genes in pancreatic β cells. *Mol. Cell* 7:559–70
62. Leibiger B, Moede T, Schwarz T, Brown GR, Kohler M, et al. 1998. Short-term regulation of insulin gene transcription by glucose. *Proc. Natl. Acad. Sci. USA* 95:9307–12
63. Leibiger B, Moede T, Uhles S, Berggren PO, Leibiger IB. 2002. Short-term regulation of insulin gene transcription. *Biochem. Soc. Trans.* 30:312–17
64. Leibiger B, Wåhlander K, Berggren PO, Leibiger IB. 2000. Glucose-stimulated insulin biosynthesis depends on insulin-stimulated insulin gene transcription. *J. Biol. Chem.* 275:30153–56
65. Leibiger IB, Leibiger B, Moede T, Berggren PO. 1998. Exocytosis of insulin promotes insulin gene transcription via the insulin receptor/PI-3 kinase/p70 s6 kinase and CaM kinase pathways. *Mol. Cell* 1:933–38
66. Leibowitz G, Oprescu AI, Uckaya G, Gross DJ, Cerasi E, Kaiser N. 2003. Insulin does not mediate glucose stimulation of proinsulin biosynthesis. *Diabetes* 52:998–1003
67. Lin X, Taguchi A, Park S, Kushner JA, Li F, et al. 2004. Dysregulation of insulin receptor substrate 2 in β cells and brain causes obesity and diabetes. *J. Clin. Invest.* 114:908–16
68. Lobner K, Steinbrenner H, Roberts GA, Ling Z, Huang G-C, et al. 2002. Different regulated expression of the tyrosine phosphatase-like proteins IA-2 and phogrin by glucose and insulin in pancreatic islets. Relationship to development of insulin secretory responses in early life. *Diabetes* 51:2982–88
69. Luciani DS, Johnson JD. 2005. Acute effects of insulin on beta-cells from transplantable human islets. *Mol. Cell. Endocrinol.* 241:88–98
70. Macfarlane WM, Smith SB, James RF, Clifton AD, Doza YN, et al. 1997. The p38/reactivating kinase mitogen-activated protein kinase cascade mediates the activation of the transcription factor insulin upstream factor 1 and insulin gene transcription by high glucose in pancreatic β-cells. *J. Biol. Chem.* 272:20936–44
71. Malaisse WJ, Malaisse-Lagae F, Lacy PE, Wright PH. 1967. Insulin secretion by isolated islets in presence of glucose, insulin and anti-insulin serum. *Proc. Soc. Exp. Biol. Med.* 124:497–500
72. Marchetti P, Lupi R, Federici M, Marselli L, Masini M, et al. 2002. Insulin secretory function is impaired in isolated human islets carrying the Gly972Arg IRS-1 polymorphism. *Diabetes* 51:1419–24
73. Martinez SC, Cras-Meneur C, Bernal-Mizrachi E, Permutt MA. 2006. Glucose regulates Foxo1 through insulin receptor signaling in the pancreatic islet β-cell. *Diabetes* 55:1581–91
74. Muller D, Huang GC, Amiel S, Jones PM, Persaud SJ. 2006. Identification of insulin signaling elements in human beta-cells: autocrine regulation of insulin gene expression. *Diabetes* 55:2835–42
75. Deleted in proof
76. Nilsson T, Schultz V, Berggren PO, Corkey BE, Tornheim K. 1996. Temporal patterns of changes in ATP/ADP ratio, glucose 6-phosphate and cytoplasmic free Ca^{2+} in glucose-stimulated pancreatic beta-cells. *Biochem. J.* 314:91–94
77. Ohsugi M, Cras-Meneur C, Zhou Y, Bernal-Mizrachi E, Johnson JD, et al. 2005. Reduced expression of the insulin receptor in mouse insulinoma (MIN6) cells reveals multiple roles of insulin signaling in gene expression, proliferation, insulin content, and secretion. *J. Biol. Chem.* 280:4992–5003
78. Ohsugi M, Cras-Meneur C, Zhou Y, Warren W, Bernal-Mizrachi E, Permutt MA. 2004. Glucose and insulin treatment of insulinoma cells results in transcriptional regulation of a common set of genes. *Diabetes* 53:1496–508
79. Okada T, Liew CW, Hu J, Hinault C, Michael MD, et al. 2007. Insulin receptors in beta-cells are critical for islet compensatory growth response to insulin resistance. *Proc. Natl. Acad. Sci. USA* 104:8977–82
80. Otani K, Kulkarni RN, Baldwin AC, Krutzfeldt J, Ueki K, et al. 2004. Reduced β-cell mass and altered glucose sensing impair insulin-secretory function in βIRKO mice. *Am. J. Physiol. Endocrinol. Metab.* 286:E41–49

81. Patel YC, Amherdt M, Orci L. 1982. Quantitative electron microscopic autoradiography of insulin, glucagon, and somatostatin binding sites on islets. *Science* 217:1155–56
82. Persaud SJ, Asare-Anane H, Jones PM. 2002. Insulin receptor activation inhibits insulin secretion from human islets of Langerhans. *FEBS Lett.* 510:225–28
83. Pipeleers D, Kiekens R, In't Veld P. 1992. Morphology of the pancreatic β-cell. In *Insulin. Molecular Biology to Pathology*, ed. FM Ashcroft, SJH Ashcroft, pp. 5–31. New York: Oxford Univ. Press
84. Pomplun D, Florian S, Schulz T, Pfeiffer AF, Ristow M. 2007. Alterations of pancreatic beta-cell mass and islet number due to Ins2-controlled expression of Cre recombinase: RIP-Cre revisited; part 2. *Horm. Metab. Res.* 39:336–40
85. Porksen N. 2002. The in vivo regulation of pulsatile insulin secretion. *Diabetologia* 45:3–20
86. Porzio O, Federici M, Hribal ML, Lauro D, Accili D, et al. 1999. The Gly972->Arg amino acid polymorphism in IRS-1 impairs insulin secretion in pancreatic β cells. *J. Clin. Invest.* 104:357–64
87. Prentki M, Nolan CJ. 2006. Islet ß cell failure in type 2 diabetes. *J. Clin. Invest.* 116:1802–12
88. Rizzo MA, Magnuson MA, Drain PF, Piston DW. 2002. A functional link between glucokinase binding to insulin granules and conformational changes in response to glucose and insulin. *J. Biol. Chem.* 277:34168–75
89. Rizzo MA, Piston DW. 2003. Regulation of β cell glucokinase by S-nitrosylation and association with nitric oxide synthase. *J. Cell Biol.* 161:243–48
90. Roper MG, Qian WJ, Zhang BB, Kulkarni RN, Kahn CR, Kennedy RT. 2002. Effect of the insulin mimetic L-783,281 on intracellular [Ca^{2+}] and insulin secretion from pancreatic β-cells. *Diabetes* 51(Suppl. 1):S43–49
91. Rothenberg PL, Willison LD, Simon J, Wolf BA. 1995. Glucose-induced insulin receptor tyrosine phosphorylation in insulin-secreting β-cells. *Diabetes* 44:802–9
92. Schatz H, Pfeiffer EF. 1977. Release of immunoreactive and radioactively prelabelled endogenous (pro)insulin from isolated islets of rat pancreas in the presence of exogenous insulin. *J. Endocrinol.* 74:243–49
93. Sesti G. 2002. Apoptosis in the beta cell: cause or consequence of insulin secretion defect in diabetes? *Ann. Med.* 34:444–50
94. Shepherd LMA, Campbell SC, Macfarlane WM. 2004. Transcriptional regulation of the IAPP gene in pancreatic β-cells. *Biochim. Biophys. Acta* 1681:28–37
95. Solinas G, Naugler W, Galimi F, Lee MS, Karin M. 2006. Saturated fatty acids inhibit induction of insulin gene transcription by JNK-mediated phosphorylation of insulin-receptor substrates. *Proc. Natl. Acad. Sci. USA* 103:16454–59
96. Spanswick D, Smith MA, Mirshamsi S, Routh VH, Ashford ML. 2000. Insulin activates ATP-sensitive K^+ channels in hypothalamic neurons of lean, but not obese rats. *Nat. Neurosci.* 3:757–58
97. Srinivasan S, Bernal-Mizrachi E, Ohsugi M, Permutt MA. 2002. Glucose promotes pancreatic islet β-cell survival through a PI3-kinase/Akt-signaling pathway. *Am. J. Physiol. Endocrinol. Metab.* 283:E784–93
98. Srinivasan S, Ohsugi M, Liu Z, Fatrai S, Bernal-Mizrachi E, Permutt MA. 2005. Endoplasmic resticulum stress-induced apoptosis is partly mediated by reduced insulin signaling through phosphatidylinositol 3-kinase/Akt and increased glycogen synthase kinase 3b in mouse insulinoma cells. *Diabetes* 54:968–75
99. Srivastava S, Goren HJ. 2003. Insulin constitutively secreted by β-cells is necessary for glucose-stimulated insulin secretion. *Diabetes* 52:2049–56
100. Stagner J, Samols E, Polonsky K, Pugh W. 1986. Lack of direct inhibition of insulin secretion by exogenous insulin in the canine pancreas. *J. Clin. Invest.* 78:1193–98
101. Suzuki R, Tobe K, Terauchi Y, Komeda K, Kubota N, et al. 2003. Pdx1 expression in Irs2-deficient mouse beta-cells is regulated in a strain-dependent manner. *J. Biol. Chem.* 278:43691–98
102. Taylor SI. 1999. Deconstructing type 2 diabetes. *Cell* 97:9–12
103. Tillmar L, Carlsson C, Welsh N. 2002. Control of insulin mRNA stability in rat pancreatic islets. Regulatory role of a 3'-untranslated region pyrimidine-rich sequence. *J. Biol. Chem.* 277:1099–106
104. Trumper K, Trumper A, Trusheim H, Arnold R, Goke B, Horsch D. 2000. Integrative mitogenic role of protein kinase B/Akt in β-cells. *Ann. N.Y. Acad. Sci.* 291:242–50
105. Tuttle RL, Gill NS, Pugh W, Lee JP, Koeberlein B, et al. 2001. Regulation of pancreatic β-cell growth and survival by the serine/threonine protein kinase Akt1/PKBα. *Nat. Med.* 7:1133–37

106. Ueki K, Okada T, Hu J, Liew CW, Assmann A, et al. 2006. Total insulin and IGF-I resistance in pancreatic cells causes overt diabetes. *Nat. Genet.* 38:583–88
107. Uhles S. 2005. *Selective insulin signaling in the pancreatic β-cell via the two insulin receptor isoforms*. PhD thesis. Karolinska Inst. 60 pp.
108. Uhles S, Moede T, Leibiger B, Berggren PO, Leibiger IB. 2003. Isoform-specific insulin receptor signaling involves different plasma membrane domains. *J. Cell Biol.* 163:1327–37
109. Uhles S, Moede T, Leibiger B, Berggren PO, Leibiger IB. 2007. Selective gene activation by spatial segregation of insulin receptor B signaling. *FASEB J.* 21:1609–21
110. Van Schravendijk CFH, Foriers A, van den Brande JL, Pipeleers DG. 1987. Evidence for the presence of type I insulin-like growth factor receptors on rat pancreatic A and B cells. *Endocrinology* 121:1784–88
111. Velloso LA, Carneiro EM, Crepaldi SC, Boschero AC, Saad MJ. 1995. Glucose- and insulin-induced phosphorylation of the insulin receptor and its primary substrates IRS-1 and IRS-2 in rat pancreatic islets. *FEBS Lett.* 377:353–57
112. Verspohl EJ, Ammon HPT. 1980. Evidence for presence of insulin receptors in rat islets of Langerhans. *J. Clin. Invest.* 65:1230–37
113. Virkamäki A, Ueki K, Kahn CR. 1999. Protein-protein interaction in insulin signaling and the molecular mechanisms of insulin resistance. *J. Clin. Invest.* 103:931–43
114. Wicksteed B, Alarcon C, Briaud I, Lingohr MK, Rhodes CJ. 2003. Glucose-induced translational control of proinsulin biosynthesis is proportional to preproinsulin mRNA levels in islet β-cells but not regulated via a positive feedback of secreted insulin. *J. Biol. Chem.* 278:42080–90
115. Withers DJ, Gutierrez JS, Towery H, Burks DJ, Ren J-M, et al. 1998. Disruption of IRS-2 causes type 2 diabetes in mice. *Nature* 391:900–4
116. Wobser H, Bonner C, Nolan JJ, Byrne MM, Prehn JH. 2006. Downregulation of protein kinase B/Akt-1 mediates INS-1 insulinoma cell apoptosis induced by dominant-negative suppression of hepatocyte nuclear factor-1alpha function. *Diabetologia* 49:519–26
117. Wolfrum C, Besser D, Luca E, Stoffel M. 2003. Insulin regulates the activity of forkhead transcription factor HNF-3b/Foxa2 by Akt-mediated phosphorylation and nuclear/cytosolic localization. *Proc. Natl. Acad. Sci. USA* 100:11624–29
118. Wu H, Macfarlane WM, Tadayyon M, Arch JRS, James RFL, Docherty K. 1999. Insulin stimulates pancreatic-duodenal homeobox factor-1 (PDX1) DNA-binding activity and insulin promoter activity in pancreatic β cells. *Biochem. J.* 344:813–18
119. Xu G, Marshall CA, Lin TA, Kwon G, Munivenkatappa RB, et al. 1998. Insulin mediates glucose-stimulated phosphorylation of PHAS-I by pancreatic beta cells. An insulin-receptor mechanism for autoregulation of protein synthesis by translation. *J. Biol. Chem.* 273:4485–91
120. Xu GG, Gao Z, Borge JPD, Jegier PA, Young RA, Wolf BA. 2000. Insulin regulation of β-cell function involves a feedback loop on SERCA gene expression, Ca^{2+} homeostasis, and insulin expression and secretion. *Biochemistry* 39:14912–19
121. Xu GG, Gao Z, Borge JPD, Wolf BA. 1999. Insulin receptor substrate 1-induced inhibition of endoplasmic reticulum Ca^{2+} uptake in β-cells. Autocrine regulation of intracellular Ca^{2+} homeostasis and insulin secretion. *J. Biol. Chem.* 274:18067–74
122. Xu GG, Rothenberg PL. 1998. Insulin receptor signaling in the β-cell influences insulin gene expression and insulin content. Evidence for autocrine β-cell regulation. *Diabetes* 47:1243–52
123. Xuan S, Kitamura T, Nakae J, Politi K, Kido Y, et al. 2002. Defective insulin secretion in pancreatic β cells lacking type 1 IGF receptor. *J. Clin. Invest.* 110:1011–19
124. Yarden Y, Ullrich A. 1988. Growth factor receptor tyrosine kinases. *Annu. Rev. Biochem.* 57:443–78
125. Zawalich WS, Zawalich KC. 2000. A link between insulin resistance and hyperinsulinemia: inhibitors of phosphatidylinositol 3-kinase augment glucose-induced insulin secretion from islets of lean, but not obese, rats. *Endocrinology* 141:3287–95
126. Zawalich WS, Zawalich KC. 2002. Effects of glucose, exogenous insulin, and carbachol on C-peptide and insulin secretion from isolated perifused rat islets. *J. Biol. Chem.* 277:26233–37
127. Zick Y. 2004. Uncoupling insulin signalling by serine/threonine phosphorylation: a molecular basis for insulin resistance. *Biochem. Soc. Trans.* 32:812–16

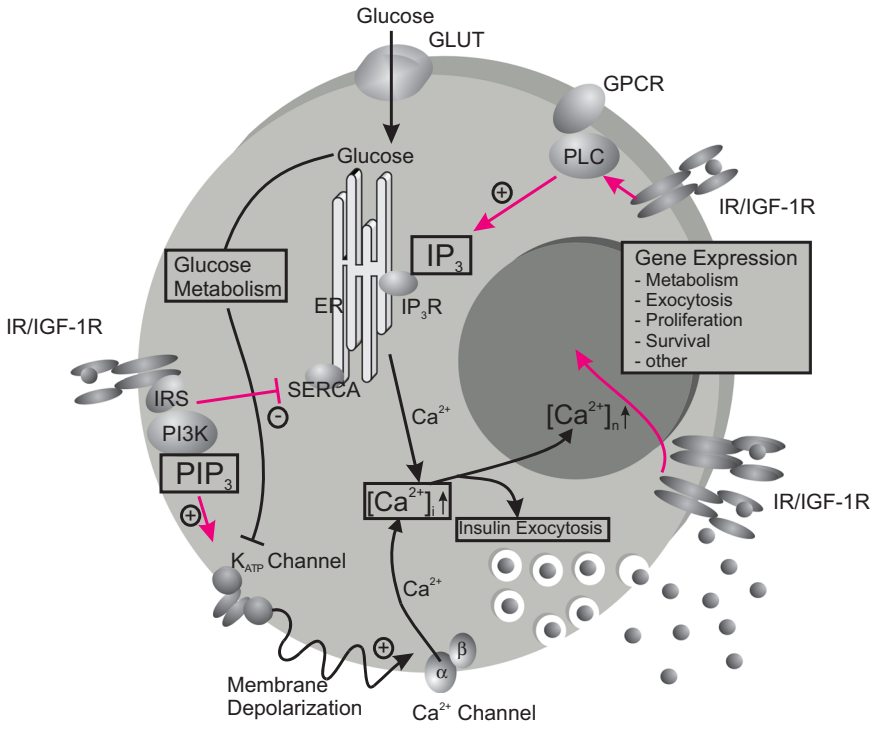

Figure 1

Scheme illustrating signal-transduction pathways involved in the β-cell stimulus-secretion coupling and their modulation by insulin feedback. $[Ca^{2+}]_i$, cytoplasmic-free Ca^{2+} concentration; $[Ca^{2+}]_n$, nuclear-free Ca^{2+} concentration; ER, endoplasmic reticulum; GLUT, glucose transporter; GPCR, G-protein-coupled receptor; IGF-1R, IGF-I receptor; IP_3R, $Ins(1,4,5)P_3$-receptor; IR, insulin receptor; IRS, insulin receptor substrate; PIP_3, $PI(3,4,5)P_3$; PLC, phospholipase C; SERCA, sarco/endoplasmic Ca^{2+} ATPase.

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Errata

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