Minireview

Insulin feedback action on pancreatic β-cell function

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Abstract Pancreatic β-cell function is essential for the regulation of glucose homeostasis and its impairment leads to diabetes mellitus. Besides glucose, the major nutrient factor, inputs from neural and humoral components and intraislet cell–cell communication act together to guarantee an appropriate pancreatic β-cell function. Data obtained over the last 5 years in several laboratories have revitalized a controversial concept, namely the autocrine feedback action of secreted insulin on β-cell function. While, historically, insulin was 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 and β-cell survival.

Key words: Insulin; Insulin receptor; Insulin secretion; Gene expression; Diabetes mellitus

1. Introduction

In adult mammals, β-cells of the pancreatic islets of Langerhans are the only source for the peptide hormone insulin and therefore these cells are of vital importance in maintaining blood glucose homeostasis. β-cells not only produce insulin but secrete the hormone in amounts appropriate to the blood glucose concentration in order to keep blood glucose levels within narrow limits. Dysfunction of pancreatic β-cells is a major cause of the development of so-called non-insulin-dependent diabetes or type 2 diabetes mellitus, the most common metabolic disorder in man.

Multiple signals of different origin guarantee appropriate β-cell function under both basal and glucose-stimulated conditions. These signals include humoral factors (hormones, vitamins, nutrients, ions, etc.), nerve stimulation, as well as factors of intraislet cell–cell communication. Whereas the paracrine effects on β-cells of glucagon, secreted from pancreatic α-cells and stimulating insulin release, and of somatostatin, secreted from δ-cells and inhibiting insulin release, are well accepted (for review see [1]), the autocrine effect of secreted insulin on β-cell function was and still is a matter of debate. Although the idea of an autocrine feedback by insulin is not new and dates back to the 1940s [2], both conceptual disagreement and different results in the respective experiments contribute to this still ongoing controversy. With regard to the conceptual disagreement, the major argument is that β-cells are exposed to so much insulin that the respective signal transduction pathways must be desensitized. Experimentally, with regard to the effect of insulin upon insulin secretion for example, all possible outcomes like negative feedback [3–8], positive feedback [9–11], and no effect at all [12–15] have been reported. Moreover, while historically insulin was exclusively discussed as a negative signal [2–7,16], recent data provide evidence for a positive role of insulin in several cellular processes that include the regulation of gene transcription [17–23], translation [18,24–26], Ca^{2+} flux [9–11,27,28], insulin secretion [9–11] and the potential role of insulin action in β-cell survival [29]. One of the major points discussed as a source for controversial results and conceptual disagreement was the question whether the observed insulin effect upon β-cell function is a direct one or rather secondary, mediated by factors of non-β-cell origin. This mainly concerned experiments on whole animals and perfused pancreata, but also the 'artificial diffusion effect' in studies on isolated islets, i.e. insulin coming from outside and thus going the wrong way, was discussed.

The aim of the present review is to summarize both historical and recent data on insulin feedback, here focusing on the two most controversially discussed areas, i.e. insulin expression/content and insulin secretion.

2. Insulin signaling in β-cells – the components

The first step in the insulin signaling cascade is binding of insulin to the insulin receptor (IR) [30]. However, because pancreatic β-cells are surely exposed to insulin concentrations that are higher than those in the periphery [31], insulin-like growth factor (IGF)-I and IGF-II receptors, which have a lower affinity for insulin [32], cannot be excluded as targets for insulin binding. That β-cells are targets for insulin was shown already in the 1980s in conventional radioligand binding assays [33] as well as by quantitative electron microscopic autoradiography [34]. The presence of IR and IGF receptors in insulin-producing cell lines was reported in [35] and [36,37], respectively. Although recent data show that all four insulin receptor substrate proteins, i.e. IRS-1, -2, -3 and -4, are detectable in pancreatic β-cells [38] and that downstream located effector proteins, such as phosphoinositide 3′-kinase (PI3 kinase), PKB/Akt, p70s6k, PHAS-1 can be activated by glucose or direct insulin stimulation [17,19,21,24], it was a major

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breakthrough when Rothenberg et al. [39] and Velloso et al. [40] in 1995 reported that insulin, secreted upon glucose stimulation, activated the β-cell IR and the downstream located IRS and PI3 kinase. These studies provided evidence for an autocrine feedback action of insulin at the molecular level but did not yet resolve whether insulin is a negative, positive or complex (negative and positive) signal in β-cell function.

3. Insulin and insulin biosynthesis, insulin content and β-cell mass

To our knowledge the first note on a potential negative feedback action of insulin regarded insulin biosynthesis/insulin content. In 1941 Best and Haist [2] reported that daily injection of rats with insulin led to a reduced pancreatic insulin content. The effect of administered insulin is in agreement with later reports on chronic administration of insulin or the effect of transplanted insulinomas (see [16]), all suggesting that insulin is a negative regulator of β-cell mass/insulin content. However, chronic administration of insulin is associated with hypoglycemia, a condition resulting in reduced insulin biosynthesis. To circumvent this problem, Koranyi et al. [16] combined hyperinsulinemic clamps with glucose clamps. Again, this study employing a 12-h insulin infusion at fixed glucose levels suggested a negative effect on insulin biosynthesis but did not reveal whether the insulin effect was a direct one. In contrast to these studies, disruption of insulin signaling in animal models at the level of IRS-2, as in the IRS-2 knockout mouse [41], or in the β-cell restricted knockout of the IR, βIRKO mouse [42], led to the reduction in pancreatic insulin content in the adult state and consequently to the development of a type 2 diabetes mellitus-like phenotype. Although a genetically engineered knockout of insulin expression led to β-cell hyperplasia in the prenatal state [43], thus supporting the idea of insulin being a negative regulator in maintaining β-cell mass, surprisingly neither the β-cell restricted knockout of the IR [42] or the IGF-IR (βIGFIRKO) [44], nor the combined general knockout of IR and IGF-IR [45] led to a change in β-cell mass prenatally, suggesting that neither insulin nor IGF-I seems to be involved in β-cell proliferation. In contrast to the βIGFIRKO mouse, βIRKO mice show an age-dependent decrease in β-cell mass which indicates a different function of the two receptors in the postnatal development of islets. However, a note of caution is necessary. Because all β-cell restricted knockouts are based on the expression of Cre recombinase under control of the insulin promoter, the possibility of the importance of the knocked-out component before the time of insulin expression (day 13 of embryogenesis [46]), thus Cre expression and generation of the knockout, cannot be ruled out.

While historically the reduction in insulin biosynthesis was the first suggestion for a possible negative feedback action of insulin [2], the increase in insulin biosynthesis was the first suggestion for a positive feedback action of the hormone [17,18]. While our data revealed that insulin, secreted upon glucose stimulation, is a key factor in glucose-dependent up-regulation of insulin gene transcription in primary rat and mouse β-cells as well as in the insulin-producing cell line HIT-T15 by signaling through IR-A/PI3 kinase/p70s6k [17], Xu and Rothenberg [18] at the same time reported that overexpression of IR in the insulin-producing cell line βTC6 led to an increase in insulin mRNA levels, insulin content and insulin secretion. Further data showed that insulin stimulates protein biosynthesis in insulin-producing cells in general [24,25] as well as insulin biosynthesis itself [18,25].

Of the mechanisms that are associated with insulin-dependent insulin biosynthesis, the best studied are those involved in the insulin-dependent stimulation of insulin gene transcription. Since in the process of insulin secretion the concentration of the hormone reaches levels much higher than observed in peripheral blood, an interesting question was whether insulin activates the transcription of its own gene by signaling through the IR or through IGF-IR. Stimulation with IGF-I did not activate insulin promoter activity nor did blocking of IGF-I receptors, using specific blocking antibodies, abolish insulin-stimulated up-regulation of insulin promoter activity [21]. On the other hand, overexpression of IRs led to a pronounced activation of the insulin promoter in response to either glucose or insulin, whereas blocking IRs by employing IR-blocking antibodies drastically reduced up-regulation of insulin promoter activity by the two stimuli [21]. The most convincing evidence for the involvement of IRs in this signaling cascade came from experiments in islets isolated from βIRKO mice [42]. Exposure of these islets to either elevated glucose concentration or exogenous insulin did not result in the up-regulation of endogenous insulin gene transcription as was the case in islets prepared from control animals [21].

The IR exists in two isoforms as a result of alternative splicing of the 11th exon (for review see [47]). The A-type (IR-A, Ex11−) lacks the 12 amino acids encoded by exon 11, whereas the B-type (IR-B, Ex11+) contains the respective amino acid sequence, which is located extracellularly at the C-terminus of the α-subunit of the receptor. Pancreatic β-cells as well as insulin-producing cell lines express both isoforms in an almost one-to-one ratio (own unpublished data). Overexpression of IR-A led to a pronounced effect of insulin stimulation on insulin promoter activation, while overexpression of IR-B did not [17,21]. More interestingly, selective blocking of B-type receptor signaling, using a blocking antibody raised against the 12 amino acids encoded by exon 11, abolished insulin-stimulated up-regulation of the glucokinase promoter but had no effect on insulin-stimulated up-regulation of the insulin promoter in the same cell [21]. This led us to suggest that insulin activates the transcription of its own gene by signaling through the A-type rather than through the B-type insulin receptor.

A general consensus exists on the involvement of PI3 kinase in glucose/insulin-dependent up-regulation of insulin gene transcription [17,19–21]. Inhibition of PI3 kinase by the pharmacological inhibitors wortmannin and LY294002 abolishes glucose- as well as insulin-induced up-regulation of insulin gene transcription [17,19–21]. The tested dose response of wortmannin and LY294002 and the effect of the dominant-negative acting mutant of the PI3 kinase class Ia adapter protein p85, i.e. Δp85, led to the conclusion that members of the PI3 kinase class Ia participate in the signal transduction. The downstream targets of PI3 kinase Ia are, however, less clear. Our own data favor the involvement of p70s6k in glucose/insulin-dependent up-regulation of insulin gene transcription. This is based on the finding that overexpression of a rapamycin-insensitive mutant of p70s6k, i.e. p70Δ2–46/ΔCT104 [48], combined with rapamycin treatment and glucose/insulin stimulation of β-cells led to levels of insulin promoter activation that were similar to those obtained following

stimulation of mock-transfected cells in the absence of rapamycin [17].

In agreement with the discussion of PDX-1 being one of the transcription factors involved in glucose-stimulated up-regulation of insulin gene transcription, Wu et al. [20] reported increased binding of PDX-1 to its binding sites in the insulin promoter in response to insulin.

4. Insulin and insulin secretion

The concept that insulin affects its own secretion is not new, however, the area is still controversial and currently under discussion. Historically, insulin secretion was suggested to be inhibited by secreted insulin [3–7], but on the other hand, similar models used by others fail to support this concept [12–14]. Most interestingly, recent data suggest that secreted insulin may have a positive effect on insulin exocytosis [9–11,27,28,42]. Aspinwall and co-workers [9] reported that secreted insulin stimulates the immediate, ongoing process of insulin exocytosis, very likely due to an insulin-dependent increase in cytosolic free Ca^{2+} ($[Ca^{2+}]_i$) [9,10].

The hypothesis that insulin signaling contributes to insulin secretion is supported by the analysis of two animal models. The βIRKO mouse, having a β-cell restricted knockout of the IR, exhibits a selective loss of glucose-stimulated acute insulin secretion and develops a diabetes type 2-like phenotype at the age of 8 weeks [42]. A similar phenotype is shown by the βIGFIRKO mouse with a β-cell restricted knockout of the IGF-1R [44]. Since the non-altered secretory response towards arginine stimulation demonstrated no failure at the level of the exocytotic machinery, the phenotype rather seems to be caused by a defect at the level of glucose sensing/metabolism and/or Ca^{2+} handling. However, care must be taken in linking the longer-term knockout effect(s) with the acute regulation demonstrated in the biochemical experiments discussed below.

As mentioned above, in some reports, feedback via the IR has been suggested to be part of a feed-forward mechanism that stimulates insulin secretion by increasing $[Ca^{2+}]_i$ [9–11,27,28]. Aspinwall and co-workers [9,10] show an insulin-stimulated increase in $[Ca^{2+}]_i$ within seconds after the 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. [11] when using the fungal insulin mimetic L-783,281, again via an IRS-1/PI3 kinase-dependent pathway. Data from Wolf's laboratory also identify IRS-1 as a key regulator in insulin-dependent β-cell Ca^{2+} homeostasis but suggest a different mechanism. The key differences in these studies are whether the rise in $[Ca^{2+}]_i$ is dependent on PI3 kinase [9–11] or not [27,28] as well as the source of the $[Ca^{2+}]_i$ rise, i.e. blockade of the endoplasmic reticulum Ca^{2+}-ATPase SERCA [27,28] or a more direct mobilization of Ca^{2+} from the endoplasmic reticulum [10,11]. These controversies, however, may be explained by the different experimental set-up. While in [9–11] only a 30-s insulin stimulus had been used, the data described in [27,28] were obtained after a 72-h exposure to elevated insulin.

A possible mechanism for the positive involvement of insulin signaling at the level of glucose sensing/metabolism is the insulin-dependent regulation of the 'glucose sensor' glucokinase. Data in support of this model show that glucose-dependent transcription of the glucokinase gene in pancreatic β-cells requires insulin signaling via IR-B/PI3 kinase class IIα-like activity and very likely PKB/Akt [21]. More interestingly, Rizzo et al. [49] recently reported that the recruitment of active glucokinase molecules from the inactive pool is insulin-dependent. Because of the slow kinetics of the insulin-dependent recruitment of activated glucokinase molecules, i.e. several minutes, this particular mechanism is unlikely to be involved in the immediate effect of insulin on the first phase of secretion as reported in [9–11], which takes place within seconds after exocytosis. However, the long-term effect of the lack of insulin-dependent regulation of glucokinase protein expression and enzyme activation may partly explain the abolished glucose-dependent first-phase secretory response observed in the βIRKO and βIGFIRKO mouse models [42,44].

Noteworthy, also the recent observations do not settle the old dispute. Besides the reported positive effect of insulin on insulin secretion there are new arguments in support of an immediate negative feedback [5,8]. These latter studies show that a further possible mechanism for insulin to regulate its secretion is to modulate the activity of the K_{ATP} channel. In neurons it has been suggested that stimulation by insulin via the activation of PI3 kinase and the production of phosphatidylinositol 3,4,5-trisphosphate ($PI(3,4,5)P_3$) can serve to open the K_{ATP} channel [50]. If this were to occur in the β-cell, the stimulus–secretion coupling should be switched off. In parallel work on a rat insulinoma β-cell model (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 [51]. However, a recent study on a more physiologically relevant model, normal mouse β-cells, has produced evidence that K_{ATP} channels can be opened by insulin via PI3 kinase [5], the most likely candidate being $PI(3,4,5)P_3$. Both phosphatidylinositol 4,5-bisphosphate and $PI(3,4,5)P_3$ have been suggested to be physiological 'openers' of K_{ATP} channels, thereby shifting the ATP-dependent closure of the channel into the physiological range of ATP concentrations [52]. The aforementioned studies of normal mouse β-cells have demonstrated a PI3 kinase-dependent inactivation of insulin secretion by hyperpolarization of the plasma membrane [5], the latter providing the potential molecular basis for pulsatile insulin release [53] via the probable production of $PI(3,4,5)P_3$.

Moreover, there are data showing an increased glucose-stimulated insulin release after inhibition of PI3 kinase or in islets of $p85^{-/-}$ mice [6,15,54,55], indeed suggesting that insulin has a negative effect on β-cell stimulus–secretion coupling. Interestingly, Eto et al. [54] report that inhibition of PI3 kinase did not interfere with the secretion-triggered pathway including glucose oxidation, ATP content or $[Ca^{2+}]_i$, but suggest the effect to be distal to the increase in $[Ca^{2+}]_i$. It is noteworthy, however, that in the mentioned reports the inhibition of PI3 kinase led to an increase only in the second phase of insulin release. Finally, recent data by Zawalich and Zawalich [15] suggest that perifusion of isolated rat islets for 45 min with exogenous insulin has neither a positive nor a negative effect on endogenous insulin secretion.

5. Concluding remarks

The aim of this review was to summarize old and new data of insulin feedback action on β-cell function with special emphasis on insulin biosynthesis/content and insulin secretion

Table 1
Effect of insulin on β-cell function

Biological function	Effect	Cell or tissue type	Signaling	Reference
Transcription				
insulin gene	down-regulation	rat islets	?	[1]
insulin gene	no effect	rat islets		[56]
insulin gene	up-regulation	HIT, islets (rat, ob mouse)	IR-A/PI3K-Ia/p70s6k	[17,21,22]
insulin gene	up-regulation	MIN6	PI3K-Ia	[19]
insulin gene	up-regulation	MIN6, human islets	PDX1-binding	[20]
insulin gene	up-regulation	βTC6-F7/IR	?	[18]
glucokinase gene	up-regulation	HIT, islet (rat, ob mouse)	IR-B/PI3K-II/PDK1/PKB	[21,22]
acetyl-CoA carboxylase gene	up-regulation	MIN6	SREBP1c	[23]
Translation				
general	up-regulation	RINm5F, rat islets	PHAS-1	[24]
general, insulin	up-regulation	rat islets	?	[25]
general, insulin	up-regulation	βTC6-F7/IR	?	[18]
IA-2	up-regulation	rat and human islets	?	[26]
Insulin content	negative	various models	?	[2,16] and references in [16]
	positive	βTC6-F7/IR	?	[18]
	positive	IRS2 KO mouse	IRS2	[41]
	positive	βIRKO mouse		[42]
Insulin secretion	negative	various models	?	[3–7]
	no effect	various models		[12–14]
	positive	mouse islets, $IRS^{-/-}$ cells	PI3K/IRS1, SERCA?	[10,11]
	positive	βTC6-F7/IR, rat islets	IRS1, SERCA	[27,28]
	negative	mouse islets	PI3K	[5]
	negative	human islets	PI3K	[8]
	no effect	rat islets		[15]
	positive	βIRKO mouse	?	[42]
	negative	$p85^{-/-}$ mouse islets	PI3K Ia	[54]
Cytosolic free Ca^{2+}	increase	mouse islets, $IRS^{-/-}$ cells	PI3K/IRS1, SERCA?	[10,11]
	increase	βTC6-F7/IR, rat islets	IRS1, SERCA	[27,28]
Proliferation	negative	insulin KO mouse		[43]
	no effect	βIRKO mouse		[42]
	no effect	βIGFIRKO mouse		[44]
	no effect	IR-IGF1R KO mouse		[45]

(see Table 1). Whereas historically insulin was discussed as a negative signal [3–7] or having no effect [12–14] on β-cell function, recent data provide evidence for a positive role of insulin in several cellular processes that include the regulation of gene transcription [17–23], translation [18,24–26], Ca^{2+} flux [9–11,27,28], insulin secretion [9–11,27,28] and the potential role of insulin action in β-cell survival [29]. Interestingly, also the recent data do not settle the old dispute but instead provide further contradictory results on insulin biosynthesis [18,25,56] and exocytosis [5,8–11,15].

However, as contradictory as these reports might look at first glance, perhaps the different experimental conditions used may offer the explanation for the reported results. One crucial aspect may be the dynamics of events triggered by insulin. In fact, secreted insulin activates its own receptor and thereby PI3 kinase rapidly. This may indeed form the basis for an immediate, i.e. within seconds to a few minutes, influence on even the first phase of insulin exocytosis, as reported in [5,8–11], very likely mediated by IRS and/or PI3 kinase products. The next level of insulin action may regulate processes within several minutes, such as gene transcription [17,19–21,23], and translation [24–26]. These processes will involve the activation of signal transduction downstream of PI3 kinase and thus trigger protein phosphorylation/dephosphorylation, protein translocation, protein–protein interaction and protein–DNA interaction, but will not require the synthesis of new proteins. This level of insulin action may also include the modulation of the first as well as the second phase of insulin secretion, the latter by promoting recruitment of secretory granules to the plasma membrane. Longer-term effects of insulin feedback action, i.e. after 30 min, may be achieved by the synthesis of proteins, including transcription factors, that regulate expression of further 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 leads to the activation (i.e. tyrosine phosphorylation by the IR) and later inactivation (i.e. serine phosphorylation by insulin-activated atypical protein kinase C) of IR and IRS [57].

Another explanation for the controversy in results may come from the mode of exposure of β-cells to insulin. In physiology, i.e. under basal as well as stimulated conditions, pancreatic β-cells secrete insulin in a pulsatile manner [53]. 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, perhaps even more important, this pulsatile insulin release may be the guarantee that pancreatic β-cells are responsive 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 insulin secretagogues, may indeed result in a negative feedback or even in a lack of response, due to the desensitization of the signaling cascade. Interestingly, loss of pulsatile insulin release is already observed in the early stages of type 2 diabetes [53].

Taken together, a growing body of evidence demonstrates

that autocrine insulin feedback, resulting in both positive and negative effects, is involved in regulating proper β-cell function. However, it has to be stressed that under normal, physiological conditions insulin feedback takes place at a time when the pancreatic β-cells face a multiple input of signals that accompany food intake and digestion. This includes neural factors, the action of incretines, enforced intraislet cell–cell communication, elevated glucose metabolism and elevated Ca^{2+} levels in β-cells, and last but not least the potential feedback action of factors co-secreted with insulin. All these factors will, in addition to providing the basis for a complex cross-talk between various signaling pathways in pancreatic β-cell function, also affect insulin-induced signal transduction.

Future research will not only have to clarify which processes that have previously been described to be neural/incretine/paracrine/glucose-dependent, are in fact insulin-dependent, but will also have to show to what extent maintenance of β-cell function is dependent on the autocrine insulin feedback action. Thus, impairment of insulin signaling in β-cells as proposed in the 'β-cell insulin resistance' concept will lead to and/or accelerate dysfunction of the β-cell, as seen in type 2 diabetes.

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