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Direct Autocrine Action of Insulin on β-Cells: Does It Make Physiological Sense?

Christopher J. Rhodes,[1] Morris F. White,[2] John L. Leahy,[3] and Steven E. Kahn[4]

In recent years there has been a growing interest in the possibility of a direct autocrine effect of insulin on the pancreatic β-cell. Indeed, there have been numerous intriguing articles and several eloquent reviews written on the subject (1–3); however, the concept is still controversial. Although many in vitro experiments, a few transgenic mouse studies, and some human investigations would be supportive of the notion, there exist different insights, other studies, and circumstantial evidence that question the concept. Therefore, the idea of autocrine action of insulin remains a conundrum. Here we outline a series of thoughts, insights, and alternative interpretations of the available experimental evidence. We ask, how convincing are these, and what are the confusing issues? We agree that there is a clear contribution of certain downstream elements in the insulin signaling pathway for β-cell function and survival, but the question of whether insulin itself is actually the physiologically relevant ligand that triggers this signal transduction remains unsettled. *Diabetes* **62:2157–2163, 2013**

ELEMENTS OF INSULIN SIGNALING PATHWAYS IN β-CELLS ARE IMPORTANT

The insulin signal transduction pathway in pancreatic β-cells is similar to that in most other cell types (Fig. 1). We do not dispute evidence that insulin receptors are expressed in β-cells. Moreover, it is generally accepted that some elements in the "insulin signal transduction pathways" play a critical role for β-cell survival, growth, and general well-being (1–4). Perhaps the best example comes from the global insulin receptor substrate (IRS)-1 and IRS-2 knockout mouse models, in which IRS-2 was shown to play an especially vital role in the ability of β-cells to compensate for insulin resistance (5–7). These landmark studies were a catalyst to change previous thinking in the diabetes research field. Before, the predominant thought was that insulin resistance was the main cause of type 2 diabetes, but it was not widely acknowledged until the 1990s that the onset of type 2 diabetes is marked by a failure of the functional β-cell mass to meet the metabolic demand (8–10). Back then, with the realization that IRS-2 signaling in β-cells could be important, a plethora of studies blossomed to indicate certain downstream elements in IRS-2 signaling pathways also play important roles in β-cell function and survival (Fig. 1) (4,8). IRS-2 is critical because it is a highly regulated "gatekeeper" of islet β-cell homeostasis. Its expression is increased by glucose, incretins such as GLP-1, and other factors that increase cytosolic $[Ca^{2+}]_i$ and $[cAMP]_i$ in β-cells (11–13). By contrast, it can be downregulated by proinflammatory cytokines, physiological stress, and feedback inhibition of normal IRS signaling (4,14–16). It is conceivable that the relatively high expression of IRS-2 and its quick turnover in β-cells (13) may offset any need for constitutive activity of the insulin receptor, as it does in the liver (17). With a controlled upregulation of IRS-2 when β-cell compensation is needed to maintain glucose homeostasis and downregulation of IRS-2 when β-cell compensation is not needed, the responsibility for insulin itself to trigger downstream signaling in β-cells could be removed and placed more so on glucose, incretins, neuronal connections, and other more physiologically relevant regulators of β-cell function.

However, despite this growing body of evidence for the necessity of IRS-2–regulated signaling pathways in β-cells, the identity of any physiologically relevant upstream ligand/receptor interaction that triggers IRS signal transduction in β-cells in vivo has been rather unclear. Of course, the insulin/insulin receptor interaction is an attractive candidate, but there are considerations and circumstances that raise significant doubt of an autocrine effect of insulin on β-cells. These are considered below.

WHAT CONCENTRATIONS OF INSULIN ARE ISLET β-CELLS EXPOSED TO IN VIVO?

There is probably only one of two possibilities. Either the β-cells are bathing in a very high concentration of locally secreted insulin, or secreted insulin is rapidly dispensed into the circulation leaving the locality of an islet but then has to complete an entire lap around the body where it is cleared by other tissues before returning to the β-cells at a much lower concentration. The net result of these two possibilities would likely be the same. A direct autocrine biological effect of insulin on the β-cell in vivo is unlikely.

Why? Prolonged exposure to insulin, and/or high concentrations of insulin in all cells that express the insulin receptor effectively desensitizes the IRS signaling pathway downstream of the receptor as well as downregulates expression of insulin receptor itself (18). There is no reason to think the β-cell is any different. Internalization of the insulin receptor into an endosomal compartment, once insulin is bound contributes to this desensitization reducing the availability of cell-surface insulin receptors (19). Some desensitization mechanisms are normal physiological feedback inhibition aimed at preventing potentially harmful effects of prolonged exposure to insulin such as hypoglycemia and/or oncogenesis (18) (Fig. 2). Once the insulin receptor is downregulated and downstream signaling desensitized, some time without insulin is needed

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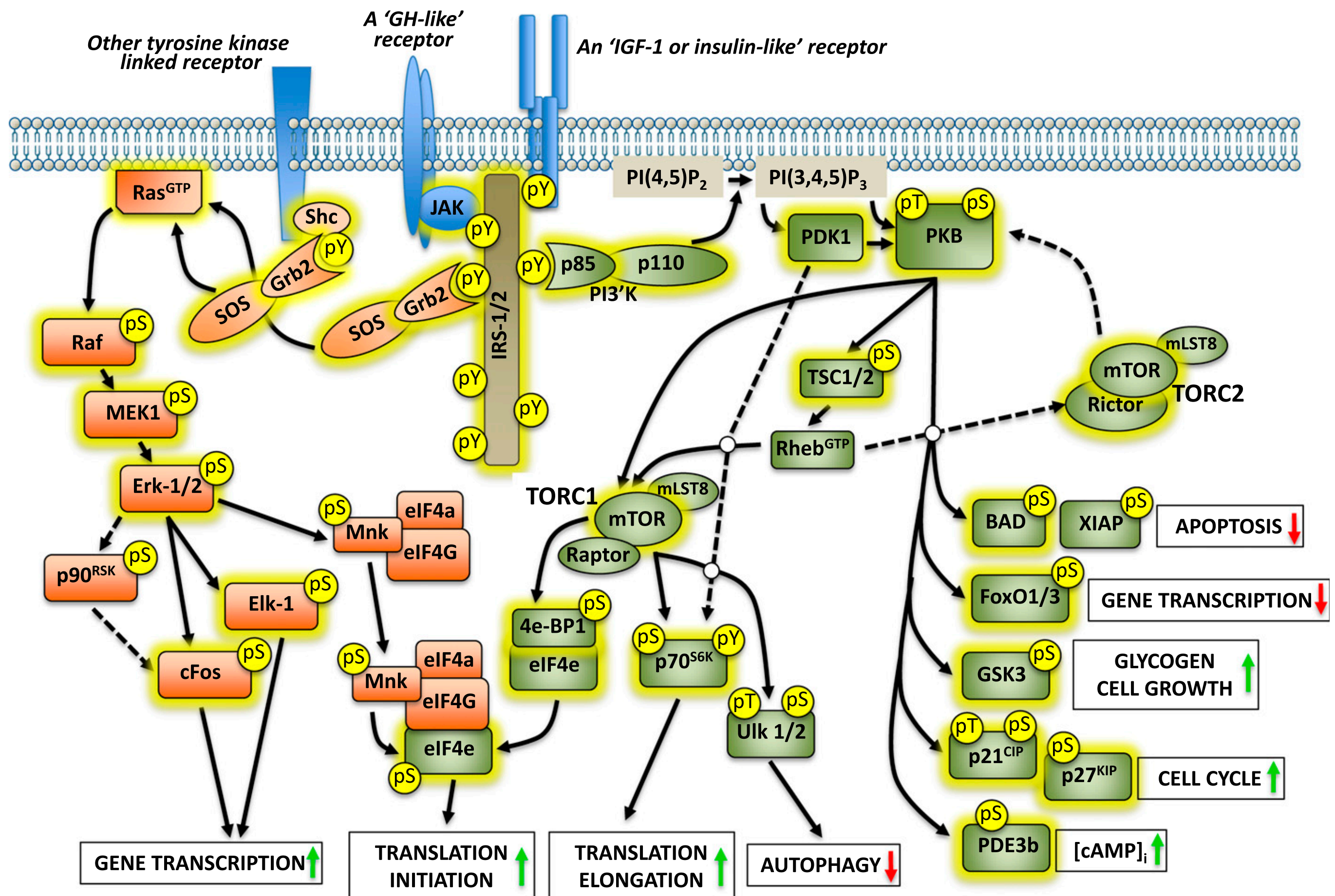

FIG. 1. Activation of the IRS signaling cascade pathways. A peptide ligand such as insulin or insulin-like growth factor-1 (IGF-1) binds to its receptor, activating the intrinsic tyrosine kinase activity of that receptor that then tyrosine phosphorylates (pY) adaptor molecules such as IRS-1 or -2. Other receptor tyrosine kinases, or receptors that activate tyrosine kinases such as Janus kinase (JAK), can also activate IRS signaling. This leads to activation of two major signaling cascades, the Ras-Raf-mitogen-activated protein kinase (MAPK) pathway (orange) and the phosphatidylinositol-3′-kinase (PI3'K)/protein kinase-B (PKB; also known as Akt) signaling pathway (green). For the Ras-Raf-MAPK pathway, growth factor receptor–bound protein-2 (Grb2)/son of sevenless (SOS) protein complex binds to specific phosphorylated tyrosines on IRS-1/2, activating the GTP/GDP exchange activity of SOS, which loads $p21^{Ras}$ (Ras) with GTP to activate Ras, leading to phosphorylation of the serine/threonine protein kinase Raf-1, which then phosphorylates the mitogen-activated protein kinase kinase (MEK1), which is then activated to phosphorylate the extracellular signal–regulated kinases-1 and -2 (Erk-1/2). Phospho-activated Erk-1/2 can then directly (or indirectly via phospho-activation of other kinases such as p90 ribosomal serine kinase [$p90^{RSK}$]) serine/threonine phosphorylate certain transcription factors, such as cFos and E-twenty-six–like transcription factor 1 (Elk-1), to upregulate gene transcription. Phospho-activated Erk-1/2 can also phosphorylate MAPK–interacting kinase (Mnk) 1 and 2, leading to phosphorylation activation of the eukaryotic initiation factor-4e (eIF4e) in a complex also containing eIF4a and eIF4G to increase general protein synthesis at the level initiation phase of translational control. For the PI3'K/PKB signaling pathway, the p85 regulatory subunit of PI3'K docks to other specific phosphorylated tyrosine sites on IRS-1/2 that then activates its p110 catalytic activity. This catalyzes the phosphorylation of phosphatidylinositol-4, 5-bisphophaste [$PI(4,5)P_2$] to phosphatidylinositol-3, 4, 5-trisphophaste [$PI(3,4,5)P_3$], which then activates 3-phosphoinositide dependent protein kinase-1 (PDK1). PDK1 then threonine (pT) phosphorylates PKB for PKB activation, which can be amplified by serine phosphorylation (pS) of PKB by the target of rapamycin complex-2 (TORC2; which includes the protein kinase, mammalian target of rapamycin [mTOR] and associated proteins rictor and mLST8). PKB has a plethora of serine/threonine phosphorylation substrates. PKB-mediated phosphorylation of the tuberous sclerosis protein-1/2 complex (TSC1/2) inhibits its GTPase activating protein activity to then load the Ras homolog enriched in brain (Rheb) protein with GTP ($Rheb^{GTP}$), leading to activation of the TORC1 (which includes mTOR and associated proteins raptor and mLST8). TORC1 can then serine/threonine phosphorylate a series of substrates. This includes the eIF4e-binding protein-1 (4e-BP1) that releases it from eIF4e binding, enabling eIF4e to associate with eIF4a and eIF4G in a complex with Mnk, where Mnk then phosphorylates eIF4e to increase rates of protein synthesis translation. This also shows how the Ras/Raf/Erk and PI3'K/PKB signaling pathways can coordinate to give a tight translational control of protein synthesis. TORC1 can also phosphorylate and subsequently activate p70 S6-ribosomal kinase ($p70^{S6K}$), which can lead to an increase in the elongation phase of protein synthesis translation. PDK1 can threonine phosphorylate $p70^{S6K}$ to amplify this effect. TORC1 also phosphorylates Unc-51–like kinases-1/2 (ULK-1/2; also known as autophagy gene-1), which results in inhibition of autophagy. Among PKB's other phosphorylation substrates are proteins involved in the apoptotic process such as Bcl-antagonist of cell death (BAD) and X-linked inhibitor of apoptosis protein (XIAP), outlining a mechanism whereby PKB is antiapoptotic. PKB phosphorylation of the transcription factors FoxO1 and FoxO3a causes their removal from the nucleus and promotes their degradation, causing an inhibition of FoxO1/3a-mediated transcription. Phosphorylation of glycogen synthase kinase-3 (GSK3) by PKB inhibits GSK3 activity, resulting in increased glycogen deposition and cell growth. Under certain circumstances, PKB can also influence increases in cell growth by phosphorylating the cell-cycle inhibitor proteins p21 cyclin-dependent kinase inhibitor-1 ($p21^{CIP}$) and p27 cyclin-dependent-kinase inhibitor ($p27^{KIP}$). PKB can also phosphorylate-inhibit phosphodiesterase-3b (PDE3b) to elevate intracellular cAMP ([cAMP]i) levels. Many of these IRS signaling elements have been shown to be expressed and active and play important roles in pancreatic β-cells in terms of certain functions, growth, and survival (rev. in 2–4), and these are indicated by a yellow highlighted halo.

to restore normal insulin sensitivity (20,21). Considering that the insulin receptor is ubiquitously expressed, such feedback inhibition mechanisms provide a dynamic means of locally controlling insulin receptor activity rather than a more complex regulation of controlling insulin receptor gene expression in a tissue-specific manner. Indeed, some believe that the chronic hyperinsulinemia found in obesity and obesity-linked type 2 diabetes can make a major

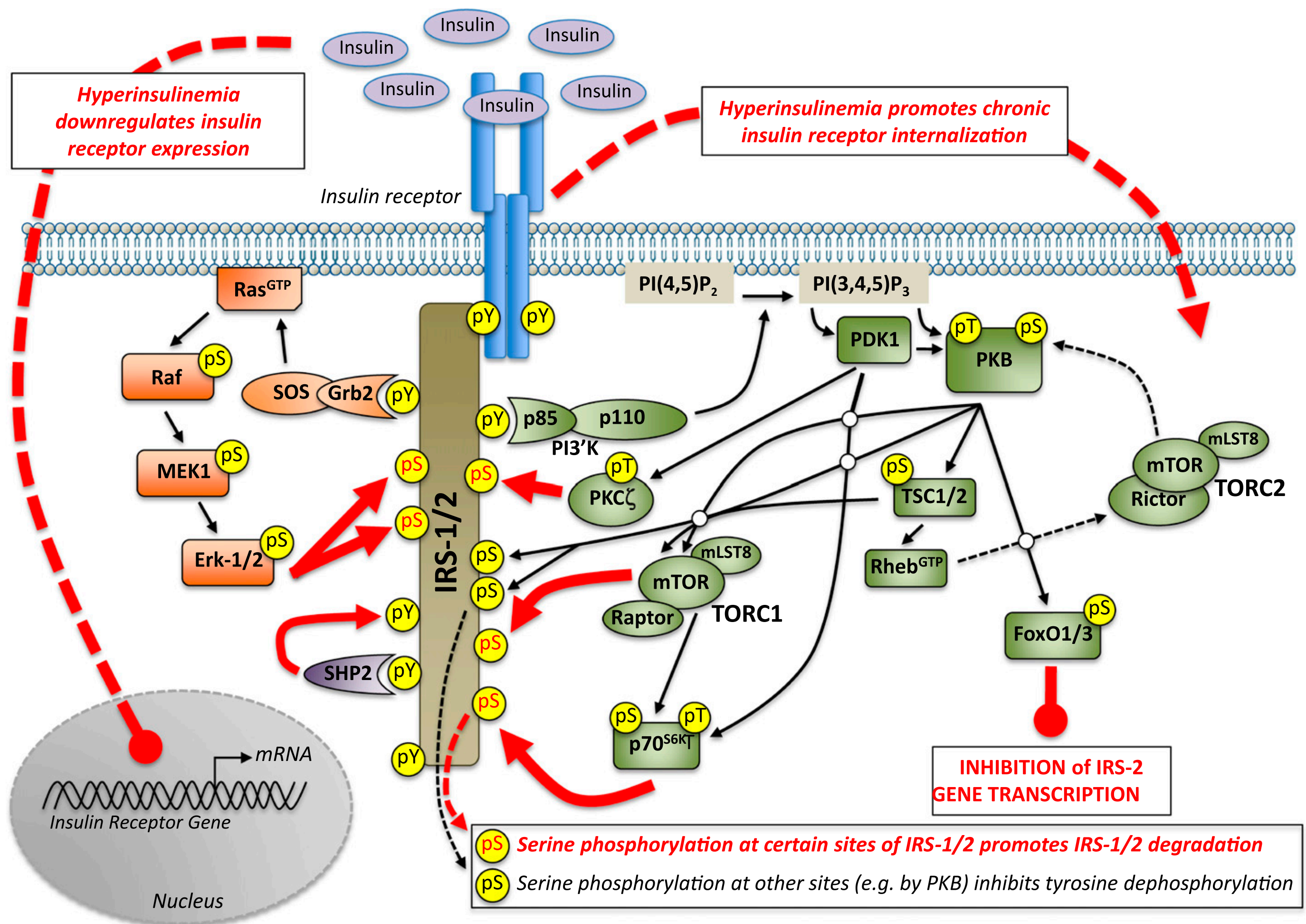

FIG. 2. Feedback inhibition of IRS signaling cascade pathways. Once insulin has activated IRS signal transduction pathways in cells, after a period of time there are internal physiological feedback inhibition signals (indicated by red arrows) that ensure that the "insulin signal" is not chronically sustained. Under hyperinsulinemic conditions, this feedback results in chronic desensitization of IRS signal transduction and contributes to the insulin-resistant state. Downstream activation of extracellular signal–regulated kinases-1 and -2 (Erk-1/2) (as described in Fig. 1) can lead to Erk-1/2 protein kinase–mediated serine phosphorylation (pS) of IRS-1/2, which results in dissociation of the insulin receptor and IRS-1/2 interaction together with IRS-1/2 degradation. This is one route of delayed feedback inhibition of insulin signaling. 3-Phosphoinositide-dependent protein kinase-1 (PDK1) activation can result in downstream activation atypical protein kinase-C (PKC) isoforms, such as PKC$_\zeta$, which can also serine phosphorylate (pS) IRS-1/2 to promote their degradation, representing another route of delayed feedback inhibition for insulin signal transduction. In contrast, protein kinase-B (PKB; also known as Akt) can serine phosphorylate IRS-1/2 at alternative sites to stabilize IRS-1/2 tyrosine phosphorylation state and thus enhance downstream signaling. However, PKB-mediated phosphorylation activation of some of its other substrates can have a more dominant-negative feedback effect on IRS signaling. Both target of rapamycin complex-1 (TORC1) and p70 S6-ribosomal kinase (p70^{S6K}) (the latter amplified by PDK1 phosphorylation) can serine phosphorylate IRS-1/2 to promote their degradation, which then dampens IRS signaling. This denotes a third route for delayed feedback inhibition of insulin signaling. A fourth route may be via the Src-homology domain–tyrosine phosphatase-2 (SHP2), which upon binding to certain phosphotyrosine residues on IRS-1/2 becomes activated and can then remove phosphate from phosphotyrosines on IRS-1/2, thus dampening downstream signaling. FoxO1 and -3a have been shown to be critical factors for driving IRS-2 expression under basal conditions, especially FoxO3a in β-cells (15). But when IRS signaling is triggered by insulin, FoxO1/3a transcriptional activity is inactivated, resulting in another route of temporal feedback inhibition by decreasing IRS-2 expression. Several of these IRS signaling feedback mechanisms have indeed been shown to be present in pancreatic β-cells (2–4,15,16). Another consideration for feedback inhibition of insulin action is that when insulin binds to its receptor, the insulin/insulin receptor complex is internalized into the cell where it dissociates in an endosomal compartment, allowing the "free" insulin receptor to return to the surface (18,19). When insulin levels are high, this cycle is biased toward there being minimal insulin receptors on the surface of the cell with the majority being internalized, and acts as an additional physiological mechanism to prevent prolonged activation of IRS signal transduction by insulin. Under chronic hyperinsulinemia, insulin receptor internalization makes a contribution to insulin resistance. This long-term hyperinsulinemia also leads to downregulation of insulin receptor gene expression by a mechanism not yet well defined.

contribution to the insulin-resistant state because of these desensitization mechanisms (18) (Fig. 2).

The concentration of insulin secreted from the pancreas in vivo in response to physiological stimuli has been estimated by measuring insulin concentrations in the portal vein under normal circumstances and can reach high peak concentrations of ≥5 nmol/L (22). As such, it is not too far-fetched to assume that if insulin were not efficiently cleared from the local islet milieu its concentration would be at least equally as high, if not higher. Considering that significant insulin receptor downregulation in insulin-targeted primary cells has been observed at lower insulin concentrations of ≤1 nmol/L (23), it is quite possible that the insulin receptor in β-cells may be permanently downregulated if insulin is not effectively cleared. In support of this idea, recent in vivo studies in normal mice have indicated that despite a marked effect of insulin in the liver to activate molecular targets, such as sterol regulatory

element binding protein-1c, there is a negligible, if any, effect of insulin on islet β-cells in the very same animal at the same time (15).

However, an alternative view is that secreted insulin is rapidly cleared from the islet locale. In favor of this scenario is the fact that pancreatic islets have an extensive microcirculatory network that is required for normal β-cell secretory function. In rodents, the islet microcirculation favors blood flow away from β-cells passing by the other pancreatic endocrine islet cell types (including glucagon-producing α-cells and somatostatin δ-cells) on exiting the islet (24). It is thought by some that this unidirectional β-cell → α-cell → δ-cell communication may be involved in coordinate downregulation of glucagon secretion as well as preventing local accumulation of somatostatin to inhibit insulin and glucagon secretion. Although human islet cell architecture is different from that in rodents, the same directional plumbing of the islet microcirculatory network is retained in humans (25). Islet β-cells are also polarized, which enables them to effectively secrete insulin into the venous islet microcirculation from where it can be readily cleared from the islet milieu (26). If insulin is efficiently dispersed away from the islet then it will be extracted from the circulation by the whole body before returning to the islet via the pancreatic artery at low peripheral picomolar concentrations (27). Such a depleted concentration of insulin could be ineffective to transduce IRS signaling in β-cells, which needs to be at ≥50 nmol/L as indicated from in vitro experiments using primary islet β-cells (2,3).

It has been argued that there is always a degree of constitutive (unregulated) insulin secretion from normal islet β-cells, and this is continually acting as a local autocrine ligand for the insulin receptor (1–3). But this observation is derived from dedifferentiated tumorigenic β-cell lines and transfection of the preproinsulin gene to pituitary cells, and thus it is not necessarily physiologically relevant. In fact, normal primary islet β-cells efficiently sort >99% of the newly synthesized (pro)insulin to the regulated pathway and there is negligible, if any, constitutive secretion (28). Notwithstanding this, even if there were constitutive insulin secretion from β-cells, the same two scenarios described above would still apply for a negligible autocrine effect of insulin on β-cells.

INFLUENCE OF THE CENTRAL NERVOUS SYSTEM ON β-CELL FUNCTION IN VIVO

Both sympathetic and parasympathetic input from the central nervous system (CNS) can affect β-cell function (29). Positive parasympathetic neurotransmitter signals, such as acetylcholine (operating through muscarinic [M3] receptors on β-cells [30]), vasoactive intestinal polypeptide, and pituitary adenylate cyclase-activating polypeptide (31), can enter via the vagus to potentiate glucose-induced insulin secretion (30,31). Conversely, some negative effects to inhibit glucose-induced insulin secretion can be transmitted via sympathetic nerves (32). In the pancreas, the vast majority of neurons associate with pancreatic islets (33). Although it has been suggested that the neuronal connections to pancreatic islets are to endothelial cells (34), it is nonetheless clear that the CNS can have a major influence on the functional regulation on β-cells (29,32). Indeed, severing the vagus nerve can significantly impair glucose-induced insulin secretion (29). In addition, an insulin-induced feedback inhibition of glucose-induced insulin secretion found in vivo is lost when the pancreas is denervated, including in humans (35–37). It has been suggested that a prior exposure to exogenous insulin can enhance subsequent stimulation of endogenous insulin secretion by glucose in humans in vivo (38,39). Although this could be consistent with a direct "priming effect" of insulin on the β-cell, an alternative explanation could be that a prior exposure to exogenous insulin downregulates and desensitizes insulin receptor signaling in the brain (18). If so, the insulin-induced feedback inhibition of glucose-induced insulin secretion would be less effective. As a consequence, mitigating this negative CNS regulation enhances endogenous insulin secretion. Indeed, an inadequate feedback inhibition of insulin on endogenous insulin secretion has been observed in obese/insulin-resistant humans and is thought to play a role in maintaining the persistent hyperinsulinemia under such circumstances (36).

Although the CNS influence on pancreatic β-cell function is often underestimated, it is attracting renewed interest. Some investigators even suggest that endogenously produced incretins may, at least in part, exert their effect on β-cells via the CNS (40,41). Intriguingly, compensatory increases in β-cell mass and function may be somewhat controlled via the CNS (42,43). Indeed, in vagotomized rats, rates of β-cell proliferation decrease by half, which is associated with an ~80% reduction in protein kinase-B (PKB; also known as Akt) activation, a key element in IRS signaling pathways (Fig. 1) (43). This in turn suggests that the CNS may have a degree of control over IRS-2–regulated signal transduction in β-cells.

However, it is noted that direct intracerebroventricular administration of insulin into the CNS does not affect circulating insulin levels, but does suppress food intake and decrease body weight (44). Although this is an intriguing observation, it relies on a pharmacological route for insulin delivery and does not reflect the physiological route by which insulin communicates with the CNS via the circulation (44). Despite the evidence that some aspects of β-cell function can be neuronally influenced, this remains a rather gray area in terms of understanding the mechanism. Further research is needed, especially in regard to identifying the specific regions of the CNS that directly communicate to the endocrine pancreas. But in terms of understanding the possible influence of insulin itself on the β-cell in vivo, it seems that this may not necessarily be a direct autocrine effect but rather a secondary one mediated in the large part via the CNS.

CENTRAL ISSUES WITH "β-CELL–SPECIFIC" TRANSGENIC KNOCKOUT MODELS

There are now several transgenic rodent models that imply the need for many of the elements of the IRS-2 signaling pathway for β-cell function, growth and/or survival (Fig. 1) (2,3,14). But a critical question is what is the physiologically relevant ligand that activates IRS signaling pathways? Is it insulin itself? Perhaps closely related insulin-like growth factor 1 (IGF-1)? Or a combination of both? Enticing transgenic mouse models in which the insulin receptor and/or the IGF-1 receptor genes have been knocked out in an intended β-cell–specific manner have been presented (45–47). Intriguingly, deleting the insulin receptor from β-cells in this manner (βIRKO mice) results in modest glucose intolerance, elevated fasting insulin levels and impaired glucose-induced first-phase insulin secretion (45). Likewise, deleting the IGF-1 receptor by the same technical approach (βIGF1r mice) reveals a similar phenotype (46).

Cross-breeding these two mouse models deletes the receptors for insulin and IGF-1 on β-cells (βDKO mice), rendering a more severe diabetic phenotype with apparent loss of β-cell secretory capacity and function, together with elevated glucagon levels in the fed state (47). Such unregulated glucagon release would likely be a major contributor to the hyperglycemic state of these double-knockout (βDKO) animals.

These data were very intriguing at the time, but recently the technical approach used to generate these transgenic mouse models has been questioned (48,49). The short form of the rat insulin gene promoter (RIP) used to drive a Cre-recombination to generate the β-cell–specific knockout of the insulin and/or IGF-1 receptor genes is unlikely to be exclusively β-cell specific (45–47). Indeed, many of the RIPs and other β-cell transcription factor (e.g., Pdx1) gene promoters cannot be considered β-cell specific because they also drive quite significant Cre-expression in several regions of the brain, including the hypothalamus (48,49). Moreover, the RIP-Cre mice themselves display mild glucose intolerance, apparently owing to Cre-impaired first-phase insulin secretion (50). As previously outlined, the CNS can have a major influence on metabolic homeostasis, including pancreatic endocrine cell functions involving the β-cell. As such, while it is likely that any potential feedback effect of insulin and/or IGF-1 on the β-cell is blocked in these animal models, it cannot be ruled out that the primary defect driving the phenotype of the βIRKO, βIGF1r, or βDKO mice originates in the CNS and that the β-cells respond secondarily to dysfunctional CNS control (51). In this regard, there are now multiple studies in which knocking out a gene using a RIP-Cre or Pdx1-Cre transgenic approach gave an obese, hyperphagic, or metabolically altered phenotype that has been primarily attributed to deleting that gene in the brain/hypothalamus rather than the β-cell (48,49). In these instances, any apparent β-cell defect might be secondary to obesity or changes in metabolic homeostasis controlled by the CNS. However, technology has progressed, and there is now a new transgenic mouse model in which the full-length (8 kb) mouse insulin promoter drives Cre-expression, apparently in β-cells only (46). Future use of this "MIP-CreER mouse" to uniquely delete insulin and/or IGF-1 receptors in β-cells may move toward resolving some of the controversy behind autocrine action of insulin.

DOES INSULIN AFFECT INSULIN GENE EXPRESSION IN VIVO?

Yes, but not necessarily in a direct autocrine manner. In vivo it has been known for more than 70 years that administration of exogenous insulin depletes the pancreas of its insulin stores (52). This is often interpreted as a negative effect of insulin on insulin gene expression (2). However, this is more likely due to the infused insulin lowering the blood glucose to a hypoglycemic state, which is known to deplete endogenous insulin stores and downregulate insulin gene expression (53,54). Insulin gene expression is markedly downregulated under starvation conditions, but then rapidly recovers upon refeeding—an effect likely driven primarily by the circulating glucose levels (53). A glucose infusion administered to fasted rats is sufficient to drive increases in insulin gene transcription (55). However, if the glucose concentration is clamped in vivo, subsequent insulin infusion to induce hyperinsulinemia can partly reduce insulin gene expression (56), but it remains unclear whether this is a direct effect of insulin on the β-cell or one acting secondarily via the CNS. In hyperglycemic states in vivo, insulin gene expression does not appear to drastically vary at the level of the β-cells. Although insulin gene expression appears reduced in pancreata from type 2 diabetic rodent models, this can be correlated with loss of β-cell mass rather than any relation to hyperinsulinemia (57,58). It should be noted that preproinsulin mRNA levels are not only transcriptionally regulated, but also at the level of preproinsulin mRNA stability. Indeed, physiologically relevant increases in glucose stabilize preproinsulin mRNA, but this does not appear to be mediated by a local autocrine feedback of secreted insulin (59).

Recently however, there has been a series of in vitro studies using transformed β-cell lines or isolated islets to suggest, in contrast to established in vivo studies, that there is a positive effect of insulin to drive insulin gene expression (2). These in vitro studies have been complemented by experiments using islets isolated from the aforementioned βIRKO and βIGF1r transgenic mice (45–47). However, in these particular mouse models, there could well be a CNS influence on the islets of these animals which alters their β-cell mass (42,43,48,49,51) and, as previously observed, preproinsulin mRNA levels parallel these changes (57). In short, none of these in vitro studies can readily place the potential of insulin itself directly regulating its own gene expression into a proper physiological context. There is some indication that elements in the insulin signaling pathway in β-cells, such as FoxO1 are involved in influencing insulin gene expression in β-cells (4). However, whether these elements are directly controlled by an in vivo autocrine feedback action of insulin is not established.

DOES INSULIN AFFECT INSULIN PRODUCTION?

The major regulation of insulin production in normal pancreatic β-cells occurs at the translational level (60). Fluctuation in glucose concentrations is the main instigator of this control, but it can be supplemented by other nutrients and incretin peptides (60). When primary β-cells are exposed to a stimulatory glucose concentration there is a 20- to 30-min lag period (due to recruitment of preproinsulin mRNA containing polyribosome complexes to the rough endoplasmic reticulum, the site of proinsulin biosynthesis) before increases in proinsulin biosynthesis are observed, that can then reach an impressive ~10-fold increase by 60 min. Glucose-induced translational control is unique to β-cells and specifically directed at proinsulin and the biosynthesis of a subset of ~50 secretory granule proteins (60). It ensures that insulin stores in β-cells are rapidly replenished after a bout of insulin exocytosis. The molecular mechanism for specific glucose-induced translational regulation of proinsulin biosynthesis is quite distinct from that for glucose-stimulated insulin secretion (60). For example, unlike Ca^{2+}-dependent regulated insulin secretion, translational control of proinsulin biosynthesis is Ca^{2+}-independent (60). Many studies have ruled out an autocrine positive feedback of insulin to drive proinsulin biosynthesis (59–61), but some have been a proponent of this possibility (62,63). Unfortunately, studies that indicate a positive effect of insulin to stimulate proinsulin biosynthesis are questionable because proinsulin biosynthesis was either not measured directly, or if it was only during the initial 30-min lag period after introduction of the stimulus, and as such these measures are inaccurate (62,63). Without an extended incubation period up to 60 min, the mechanics for translational

control of proinsulin biosynthesis cannot be fully appreciated (60). Thus, the vast majority of evidence indicates that insulin does not have an autocrine effect on its own production in the β-cell.

DOES INSULIN AFFECT INSULIN SECRETION?

As previously noted, there is an in vivo temporal negative feedback of insulin on stimulated insulin secretion that is mediated via the CNS in humans (35–37). However, there is an intriguing suggestion that insulin may also have a positive effect to enhance its own secretion (1–3). Corroborating evidence for this has been indicated in humans in vivo (38). But an alternative explanation could be that the effect was mainly mediated via alleviating the negative feedback of insulin through the CNS (36), as previously discussed. Yet, the majority of studies arguing for a positive autocrine effect of insulin to drive insulin secretion have been conducted in vitro where central control no longer operates, or in transgenic mouse models where a primary effect of insulin in the CNS to which β-cells act secondarily cannot be ruled out (1,2,51). Thus, an in vivo positive autocrine effect of insulin on the β-cell remains questionable. But in our minds, a feed-forward positive effect of insulin on insulin secretion from the β-cell does not make sense physiologically. If this were the case, the more insulin secreted the bigger the autocrine stimulus would be for further insulin secretion, which would forever be increasing with time and eventually have serious deleterious consequences. In this scenario, it would be difficult to see how insulin secretion would be efficiently shut off to critically avoid hypoglycemia. One could consider the temporal feedback loops in insulin signaling (Fig. 2), or "natural" protective insulin resistance (18), would be expected to control this, but such built-in "off mechanisms" take time (a few hours) to be effective (20,21). In contrast, it is established that upon removal of a stimulus, such as glucose, insulin secretion returns to basal levels in minutes. As such, in the in vivo physiological context, it seems unlikely that insulin itself will modulate local insulin secretion.

FINAL THOUGHTS AND CONCLUSIONS

We have no disagreement that multiple downstream elements of the insulin signal transduction pathway are critical for normal β-cell function, growth, survival, and general well-being (Fig. 1). We believe this is established. However, our thoughts and alternative interpretations have led us to believe that autocrine action of insulin is not established, especially when considering the in vivo physiological context. If insulin is not the appropriate ligand for β-cells, then what might be the relevant one? IGF-1? We mentioned doubts about this previously, but also note that in obesity/insulin resistance, when β-cell mass and function may increase in compensation, IGF-1 binding proteins also increase, which would actually decrease the "free" effective concentration of IGF-1. So, perhaps it is not IGF-1 either. An intriguing possibility might be IGF-2, which has been proposed to be cosecreted with insulin in response to incretins, and has an "autocrine feedback" action via the IGF-1 receptor (64). However, this hypothesis is mostly based on in vitro observations and requires in vivo testing to substantiate. Moreover, a possible autocrine feedback of IGF-2 on β-cells is also subjected to the same arguments of desensitization made here for insulin, so the jury should still be out on IGF-2 for the time being. Another consideration is that a homologous ligand may not be necessary at all, and it is the tight control of IRS-2 expression in β-cells through the action of many heterologous factors that acts as a gatekeeper to control downstream β-cell homeostasis (11–17). Moreover, the CNS may drive compensatory increases in β-cell function and mass in response to obesity/insulin resistance (42,43). However, there are also a plethora of other untested growth factor ligand candidates to consider that could act on the β-cell which, via their specific receptor, could induce tyrosine kinase activity to engage IRS adapter molecules to then transduce downstream signaling (Fig. 1). Future studies may validate such other ligands.

In conclusion, the question that insulin is the physiologically relevant molecule responsible for autocrine regulation of the β-cell is still open. Doubts and issues remain that are currently difficult to answer convincingly, however as new technologies emerge these could be better addressed experimentally in the near future. But, for the moment, we prefer use of the term "IRS signal transduction/signaling pathway" rather than "insulin signal transduction/signaling pathway" in reference to elements of these signaling networks in β-cells. This will avoid the implication that autocrine action of insulin in vivo is established, when in many quarters it is suggested that the concept remains unproven.

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