Insulin signalling in islets

Shanta J. Persaud, Dany Muller and Peter M. Jones
Beta Cell Development & Function Group, King’s College London, London, U.K.

Abstract
Studies in transgenic animals, rodent insulin-secreting cell lines and rodent islets suggest that insulin acts in an autocrine manner to regulate β-cell mass and gene expression. Very little is known about the in vitro roles played by insulin in human islets, and the regulatory role of insulin in protecting against β-cell apoptosis. We have identified mRNAs encoding IRSs (insulin receptors) and downstream signalling elements in dissociated human islet β-cells by single-cell RT (reverse transcription)–PCR, and perifusion studies have indicated that insulin does not have an autocrine role to regulate insulin secretion from human islets, but activation of the closely related IGF-1 (insulin-like growth factor 1) receptors is linked to inhibition of insulin secretion. Knockdown of IR mRNA by siRNAs (small interfering RNAs) decreased IR protein expression without affecting IGF-1 receptor levels, and blocked glucose stimulation of preproinsulin gene expression. Similar results were obtained when human islet IRS (IR substrate)-2 was knocked down, whereas depletion of IRS-1 caused an increase in preproinsulin mRNA levels. Studies using the mouse MIN6 β-cell line indicated that glucose protected β-cells from undergoing apoptosis and that this was a consequence, at least in part, of insulin release in response to elevated glucose. IGF-1 also exerted anti-apoptotic effects. These data indicate that insulin can exert autocrine effects in human islets through receptors on β-cells. It protects β-cells against apoptosis and increases preproinsulin mRNA synthesis, but does not affect insulin secretion.

Introduction
Many studies have been performed over the last 40 years to define the mechanisms regulating insulin secretion from β-cells and how insulin exerts its actions in its target cells, and we now have good working models of these processes. However, rather less is known about insulin’s capacity to act in an autocrine manner to regulate β-cell function. Several in vitro studies using rodent islets and insulin-secreting cell lines indicate that insulin does have an important autocrine function. Thus mRNAs for the IR (insulin receptor) and IRS (IR substrate)-1 in rat islets have been localized to β-cells by single-cell RT (reverse transcription)–PCR [1], and exogenous insulin stimulates insulin gene expression in HIT [2,3] and MIN6 [4,5] insulin-secreting cells and rat islets [3,6]. Glucose-stimulated phosphorylation of FoxO1 (forkhead box O1), a transcription factor implicated in β-cell mass regulation, has been shown to occur through secreted insulin acting at β-cell IRSs [7] and insulin has also been implicated in regulating insulin secretion in vitro through direct autocrine effects, but these studies are more difficult to perform and interpret. There is no consensus in this area, but several carefully conducted studies suggest that insulin does not play a major feedback role to regulate its secretion from rodent β-cells [4,8,9].

Transgenic animal models have also provided detailed information about the role played by the insulin signal transduction cascade in the regulation of β-cell mass. It is clear from these studies that IRS-2 plays a pivotal role in maintaining β-cell mass to compensate for insulin resistance in Type 2 diabetes [10] and the importance of IRSs upstream of IRS-2 has been demonstrated by significant reductions in β-cell mass and islet number in mice in which the IR was selectively deleted in β-cells [11]. More recently the use of targeted knockout of IRs in the β-cell (βIRKO) and liver (LIRKO) has indicated that insulin stimulates compensatory β-cell proliferation by promoting nuclear exclusion of FoxO1 and consequent increased nuclear expression of PDX-1 (pancreatic and duodenal homeobox 1) [12]. Moreover, these studies indicated that the closely related IGF-1 (insulin-like growth factor 1) receptors were not involved in islet proliferation in response to insulin resistance [12].

Although data generated using animal models are undoubtedly of importance in helping to delineate the roles played by insulin in regulating β-cell function, interpretation may be confounded by reductions in hypothalamic, as well as β-cell, gene expression when using the rat insulin promoter, and by the possible deleterious effects of gene deletion on normal β-cell development. Furthermore, since signal cascades that have been defined in rodents are not always regulated in an identical manner in humans, it is necessary to confirm whether data generated using rodent β-cells and islets are also applicable to human islets.

Insulin signalling elements in human β-cells
The term ‘islet’ is sometimes used interchangeably with ‘β-cell’, and identification of a gene or protein in isolated
islets may be considered to indicate that these elements are actually expressed by β-cells. This may well be the case, especially in rodent islets where β-cells constitute approx. 70–80% of the islet mass, but only 50–60% of cells in human islets are β-cells [13], and obtaining human islet preparations of the >95% purity of rat and mouse islets is not easily achieved. Rat islet β-cells can be purified fairly readily by FACS, but this method is not readily adapted for human islets as they have a high intrinsic fluorescence. Thus we have used single-cell RT–PCR, in which the identities of single FACS, but this method is not readily adapted for human islets are achieved. Rat islet

Knockdown of IR and IRS mRNAs in human islets

Knockdown of IRs in the mouse MIN6 β-cell line using siRNA (small interfering RNA) demonstrated that both glucose- and insulin-stimulated PPI (preproinsulin) gene expression were decreased [4], and, consistent with this, stable IR knockdown caused significant reductions in PPI mRNA levels [5]. However, deletion of β-cell IRSs in vivo was not associated with decreased PPI mRNA [11], although pancreatic deletion of IRS-2 did reduce PPI gene expression [16]. Islets from individuals with Type 2 diabetes show reduced IR and IRS-2 mRNA expression [17], but the autocrine role of insulin in regulating PPI mRNA levels in human islets had not been examined previously. We used the siRNA-mediated transient gene-knockdown approach [4] and found that depletion of human islet IRSs inhibited glucose-stimulated PPI gene expression [14]. Furthermore, siRNA-induced silencing of IRS-1 and IRS-2 in human islets indicated that insulin regulates PPI gene transcription through IRS-2, since IR and IRS-2 knockdown significantly inhibited glucose stimulation of insulin gene expression, whereas decreased IRS-1 expression caused a significant stimulation (Figure 1). These data imply that, in human islets, IR activation by insulin, secreted in response to elevated glucose, signals through IRS-2 to up-regulate PPI mRNA levels, and that IRS-1 negatively regulates this process.

Insulin does not have an autocrine effect on insulin secretion from human islets

We have used previously an insulin-mimetic, L-783281, to examine the effects of IR activation on insulin secretion from human islets without the complication of quantifying endogenous insulin secretion following exogenous insulin application [18]. L-783281 inhibited insulin secretion, but reports using other tissues and our more recent studies indicate that L-783281 activates other receptor tyrosine kinases in addition to IRs. Thus it stimulates tyrosine phosphorylation of the Trk family of tyrosine kinase receptors through interaction with an intracellular domain [19], and we have found that exogenous insulin, even at concentrations as high as 10 μM, does not inhibit human islet C-peptide secretion (Figure 2), but IGF-1 does. It is likely that, in our earlier study [18], the inhibitory effects on insulin secretion that we observed with L-783281 were a consequence of its activation of IGF-1 receptors. Previous perfusion experiments, similar to that shown in Figure 2, have also indicated that insulin has no effect on C-peptide secretion from rat [9] or human [20] islets, and other in vitro studies using rodent islets or β-cells are consistent with insulin not having an autocrine role to regulate insulin secretion (e.g. [4,21]). Reports of insulin exerting a negative regulation of insulin output may therefore reflect its actions at β-cell IGF-1 receptors when used at high concentrations, but there is no obvious explanation of why some studies have indicated that insulin may positively regulate its own release (e.g. [6,22]).

Insulin protects β-cells against apoptosis and stimulates β-cell proliferation

Under normal circumstances, β-cell mass is maintained at levels sufficient to regulate glucose homeostasis, and it can expand under conditions of increased insulin requirement, such as during pregnancy or obesity. This expansion occurs
through either β-cell replication or neogenesis, and it may be associated with reduced β-cell apoptosis. As outlined above, insulin exerts a vital autocrine signalling role to maintain β-cell mass, since pancreatectomies from mice in which β-cell IR expression had been ablated demonstrated marked reductions in islet number and β-cell mass [11]. This was associated with significant reductions in β-cell proliferation, but there was no change in β-cell apoptosis [12,23]. Post-mortem analysis of pancreases obtained from individuals with Type 2 diabetes indicate significantly decreased β-cell volume and increased incidence of apoptosis [24], providing indirect evidence that insulin plays an anti-apoptotic role in β-cells. We have demonstrated a direct effect of insulin to decrease apoptosis in MIN6 β-cells [25]. In these experiments, both exogenous insulin and IGF-1 inhibited induction of caspase 3 activity and the protective effect of glucose in this β-cell model was dependent on its stimulation of insulin secretion. We also found that the stimulatory effects of glucose on MIN6 β-cell proliferation were a consequence of glucose-stimulated insulin secretion, and signalling through the phosphoinositide 3-kinase pathway was required for both the proliferative and anti-apoptotic effects of insulin [25].

Figure 2 | Insulin does not affect C-peptide secretion from human islets

Human islets were perfused [18] for 10 min with a buffer supplemented with 2 mM glucose, then with buffer supplemented as indicated. Fractions were collected every 2 min, and C-peptide secretion was measured by RIA.

Summary

There is convincing evidence that rodent and human β-cells express IRs through which secreted insulin acts to regulate β-cell function. Figure 3 illustrates that insulin secreted in response to elevated blood glucose levels acts in an autocrine manner to stimulate insulin gene expression and to regulate β-cell mass through a combination of enhanced proliferation and decreased apoptosis. IRs are not linked to changes in insulin exocytosis. The key autocrine roles played by insulin appear to be in ensuring that β-cells have sufficient PPI mRNA stores to allow insulin biosynthesis to exceed demand, and to maintain β-cell mass under normal physiological circumstances. When considering the consequences of insulin resistance, attention should be paid to the β-cell as well as to the classical target tissues: insulin resistance will lead to depletion of insulin stores through reduced insulin gene transcription, to reduced β-cell proliferation and to enhanced responses to apoptotic stimuli. Thus, although insulin resistance at the β-cell level will not affect regulated insulin secretion in the short term, in the longer term, reduced insulin synthesis and reductions in β-cell mass will exacerbate insulin insensitivity in target tissues and promote the development of Type 2 diabetes.

Our studies have been funded by generous support from The Eli Lilly International Foundation and Diabetes U.K. D.M. is a Diabetes U.K. RD Lawrence Research Fellow. We are grateful to Professor Stephanie Armel and Dr Guo Cai Huang (Division of Gene & Cell Based Therapy, King’s College London) for the provision of isolated human islets of Langerhans, to Dr Henry Asare-Anane for assistance with perifusions, and to Professor Ramasamyiyer Swaminathan (Department of Chemical Pathology, St Thomas’ Hospital, London, U.K.) for the C-peptide immunoassay.

References


Received 9 November 2007
DOI: 10.1042/BST03630290

©The Authors Journal compilation ©2008 Biochemical Society