HIGHLIGHT

Insulin begets insulin

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The pathogenesis of type 2 diabetes is characterized by insulin resistance, primarily in skeletal muscle, fat and liver, and a relative failure of the pancreatic β -cell (1, 2). Considerable controversy surrounds the issue of which of these deficiencies is the primary cause of diabetes. In some studies, the earliest observed defect is dysfunctional secretion and in others insulin resistance appears to be the first detectable problem. Some important new studies open a new perspective to these questions.

Glucose is the principal regulator of insulin secretion from pancreatic β -cells. Insulin is secreted immediately in response to elevated glucose concentrations. After insulin secretion, an immediate response takes place within the β -cell to replenish insulin stores through activation of insulin biosynthesis at the transcriptional (3) as well as translational levels (4, 5). The signals that govern the signal transduction pathway that link the glucose stimulus to the initiation of insulin gene transcription have been largely unclear. Intra-islet interactions as well as extrapancreatic hormones and neural inputs exert an important level of control over insulin synthesis and secretion and ultimately glucose homeostasis. Several lines of evidence support the possibility of an autocrine action of insulin on β -cells. Insulin binds to the surface of β -cells (6, 7), and functional insulin receptors and insulin receptor substrates (IRSs) identical to those found in peripheral tissues have been identified in both clonal and primary β -cells (6, 8, 9). Glucose stimulation of β -cell lines activates the β -cell insulin receptor much in the same way as application of exogenous insulin, suggesting that insulin secreted from β -cells binds to the insulin receptor eliciting a physiological response (10). The complete physiological consequences of insulin receptor activation of the β -cell have yet to be completely elucidated, but at least one effect is initiation of protein synthesis at both transcriptional and translational levels (11).

However, there remains controversy on the effects of insulin on β -cell secretion. Several reports have shown that glucose-stimulated insulin or C-peptide secretion from islets or perfused pancreas is suppressed in the presence of exogenous insulin, leading to the concept of a negative feedback of insulin secretion by insulin (12–18). Under similar conditions, however, other investigators have found little or no effect of insulin on glucose-stimulated insulin secretion (19–21). Furthermore, these data have been difficult to

interpret because of neuronal and intra-islet hormonal regulatory mechanisms that could interact with exogenous insulin, and the use of high glucose levels in these studies could *per se* evoke the effects of substantial stimulation of the β -cell insulin receptors masking the effect of exogenous insulin.

Experiments with purified β -cells have also generated conflicting evidence for insulin feedback. Glucose-stimulated insulin secretion from purified β -cell lines is inhibited by exogenous insulin levels (1 $\mu\text{mol/l}$) (21). In contrast, measurements of the effect of insulin on C-peptide secretion in βTC3 cells failed to show direct evidence of secretory regulation by insulin. Furthermore, transfected $\beta\text{TC6-F7}$ cells in which the insulin receptor was overexpressed showed enhanced basal and glucose-stimulated insulin secretion, but fractional secretory levels remained unchanged at all glucose concentrations whereas cells expressing kinase negative (inactive) insulin receptors showed decreased glucose-stimulated insulin secretion (11). These results suggest an autocrine pathway regulating one or more of the following: insulin secretion, insulin synthesis, and glucose sensing/utilization.

Several studies have revisited the question on whether insulin may have an autocrine effect on insulin synthesis and secretion.

Leibiger *et al.* (22) studied the effects on insulin gene transcription of depolarization of β -cells by adding (i) glucose, (ii) KCl, or (iii) sulfonylureas to the culture medium. The depolarization, which takes place in all three cases and induces insulin secretion, was also accompanied by an increase in insulin gene transcription as assessed by reverse transcription PCR and by transfected insulin-promoter–green fluorescent protein reporter constructs. By adding various pharmacological inhibitors of the signal transduction pathway, the investigators delineated two pathways leading to an increased insulin gene transcription rate: the PI-3 kinase/p70 s6 and the CaM kinase II. Furthermore, stimulation of hamster insulinoma (HIT) cells with glucose or insulin led to increased PI-3 kinase activity in immunoprecipitates obtained with anti-IRS-2 antibodies. These same pathways are also involved in insulin receptor signal transduction. Thus the investigators studied the effect of overexpression of type A and B insulin receptors in HIT cells and found that the overexpression of type A led to an enhanced insulin

transcription response upon glucose stimulus. Moreover, the authors mutated several critical sites within the insulin promoter and then studied the effects of extracellular stimuli on insulin gene transcription. The elements within the insulin gene promoter found to be critical for insulin-stimulated insulin transcription are DNA binding sites for β -cell-specific transcription factor PDX-1 (23, 24).

Aspinwall *et al.* (25) reported that in isolated murine, human, canine and porcine β -cells exogenous insulin elicited a secretory response, which was measured by a sophisticated amperometric measurement. For these assays, microelectrodes were positioned approximately 1 μ m from a cell and stimulant was applied from a micropipette approximately 30 μ m from the cell. When secretion by vesicle fusion occurs, a current spike is recorded and corresponds to quantitative detection of molecules released by exocytosis. In their studies they demonstrated that immunoneutralization of insulin could prevent the measured secretory response. Furthermore, the effect of exogenous insulin was also abolished in the presence of anti-insulin receptor antibody, confirming that the insulin receptor on β -cells is required for the secretory response. To test the possibility of direct autocrine actions of insulin, single β -cells were stimulated with potassium in the presence and absence of anti-insulin receptor antiserum. The results indicate that addition of antibody blocked the released insulin from further enhancing insulin release. Furthermore, there was a (non-significant) tendency towards a glucose concentration dependency of insulin-stimulated insulin release. The effects of insulin were not accompanied by depolarization of the β -cells, and insulin-stimulated secretion was not dependent upon extracellular calcium.

A remarkable *in vivo* study has been performed by Kulkarni *et al.* (26), who have generated mice with a tissue-specific knockout of the insulin receptor in pancreatic β -cells. In these β -cell insulin receptor knockout (β IRKO) mice, several features of type 2 diabetes mellitus are found. No significant changes in random baseline glucose levels were detectable in 2- or 6-month-old mice. However, a mild fasting hyperinsulinemia was detectable at 6 months of age in both male and female mice. When the mice were challenged with a glucose load, a clear change in insulin secretory response was found. In control littermates, a 3- to 4-fold increase in circulating insulin levels was observed 2 min after an i.p. application of glucose, with elevated insulin levels up to 30 min after glucose injection. In contrast, in the β IRKO animals the acute insulin response to glucose was absent (male mice) or severely blunted (female mice), and a slow increase of insulin levels followed until at 30 min the insulin levels were comparable with those of the control animals. This observation is most likely due to a disturbed first phase insulin response to glucose while the second phase appears to be intact. An arginine challenge, a second

insulin secretagogue, was unaltered in the β IRKO mice, thus suggesting that the β -cell dysfunction in β IRKO animals is specific to glucose, and that a functional insulin receptor is a prerequisite for the normal glucose-stimulated insulin secretion.

Glucose tolerance, as assessed by i.p. glucose loading, was already impaired in β IRKO animals at 2 months and continued to deteriorate as their age advanced. This was accompanied by reduced insulin levels throughout a glucose tolerance test.

Morphologically the islets appeared normal at 2 months of age in all mice. At 4 months there appeared to be a 20–40% reduction in islet size in the β IRKO mice. Whole pancreatic insulin content, as measured by RIA, was significantly reduced by about 35% in the β IRKO animals at 4 months of age. Glucose transporter 2 (GLUT2) immunoreactivity in pancreatic β -cells appeared unchanged in the β IRKO animals. In summary, these β IRKO animals exhibit an age-dependent progressive impairment in their ability to dispose of a glucose load. It would be nice to know whether, in these mice, the defect in insulin secretion from the pancreatic β -cell *per se* causes peripheral insulin resistance.

The studies make it clear that the effects of insulin are required for a normal function of the pancreatic β -cell. There are other factors such as neuronal inputs, incretins, free fatty acids, still unknown agents, and of course glucose which are required for the normal, physiological insulin secretory response.

The existence of a positive feedback of insulin on insulin secretion may allow the explanation of several phenomena in β -cells. Insulin secretion from islets has been demonstrated to be oscillatory in nature, and many models of oscillation have assumed some form of positive feedback by a diffusible factor released from β -cells (27–29). No compound has been satisfactorily identified that could serve this role. The results of the present studies indicate insulin as a possible candidate. Oscillations in insulin release are of significant interest because loss of oscillatory release is an early symptom of type 2 diabetes mellitus (30). Finally, it has been demonstrated that many type 2 diabetics have a marked reduction in first phase insulin secretion (just as the β IRKO mice) (31).

The observation that insulin receptors on β -cells mediate insulin secretion and synthesis, in addition to the well known role of activating peripheral glucose utilization, leads to the possibility of a direct link between dysfunctional insulin secretion and insulin resistance. It is now conceivable that either secretion of insulin from β -cells or insulin resistance at the β -cell level may be the first pathogenic disturbance in type 2 diabetes.

Since it is recognized that type 2 diabetes mellitus is a genetic disorder, the answer most likely lies in a collusion of several genetic variations and/or mutations. Additionally, the questions are raised whether agents such as the incretin glucagon-like peptide-I, the growth factor insulin-like growth factor-I, or other

Figure 1 Coupling of insulin secretion (extracellular insulin) with insulin gene transcription. Additionally insulin appears to have a positive effect on insulin secretion, hypothetically by increasing intracellular calcium induced by pathways downstream of the insulin receptor.

agents can partially ameliorate β -cell function if the signaling through the insulin receptor deteriorates.

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