Insulin secretion: **Feed-forward control of insulin biosynthesis?**

Guy A. Rutter

It has long been accepted wisdom that insulin secreted from islet β cells has either no effect, or an inhibitory feedback effect, on insulin synthesis and secretion. Recent work suggests, instead, that secreted insulin acts directly on β cells, via its own receptor, to enhance insulin production in an autocrine feed-forward loop.

Address: Department of Biochemistry, School of Medical Sciences, University of Bristol, Bristol BS8 1TD, UK.
E-mail: g.a.rutter@bris.ac.uk

Current Biology 1999, 9:R443–R445
http://biomednet.com/elecref/09609822009R0443

 ISSN 0960-9822

Insulin, the body's chief anabolic hormone, is synthesised in pancreatic islets, and released when the blood glucose level rises. Defective insulin release contributes to non-insulin-dependent diabetes mellitus (NIDDM). A new study has now shown that targeted inactivation of insulin receptors in pancreatic β cells, by genetic manipulation of mice using the Cre–*loxP* system, produces animals which suffer insulin secretory defects akin to those found in human patients with NIDDM [1]. Furthermore, *in vitro* studies have shown that exogenous insulin added directly to normal islets causes transcriptional up-regulation of the preproinsulin gene [2], activation of protein translational machinery [3] and insulin secretion [4]. The important implication is that insulin acts back on the β cells to promote its own production.

Insulin biosynthesis

In mammals, an increase in the blood glucose level enhances transcription of the preproinsulin gene [5] and translation of preproinsulin mRNA [6], and stimulates the release of insulin by regulated exocytosis of the mature hormone. The latter process, which resembles the regulated release of neurotransmitters from neurons, involves Ca^{2+} influx through L-type channels, an increase in intracellular Ca^{2+} concentration and exocytosis from dense-core secretory vesicles (Figure 1) [7].

Is secreted insulin involved in stimulating insulin gene expression? Certainly, the order of events in response to glucose is compatible with this idea. Insulin secretion is activated seconds to minutes after an elevation in the glucose level, preproinsulin mRNA translation is activated within minutes, and transcription of the gene is activated after about an hour [8]. Before we can conclude that insulin plays an important role in this process, however, a number of other criteria must be also be satisfied: blockade of insulin receptor function in islet β cells should impair insulin production; addition of exogenous insulin to islets incubated *in vitro* should enhance preproinsulin gene expression (through activation of transcription, translation or both); and changes in preproinsulin gene expression should parallel changes in insulin secretion.

Generation of β-cell insulin receptor knock-out mice

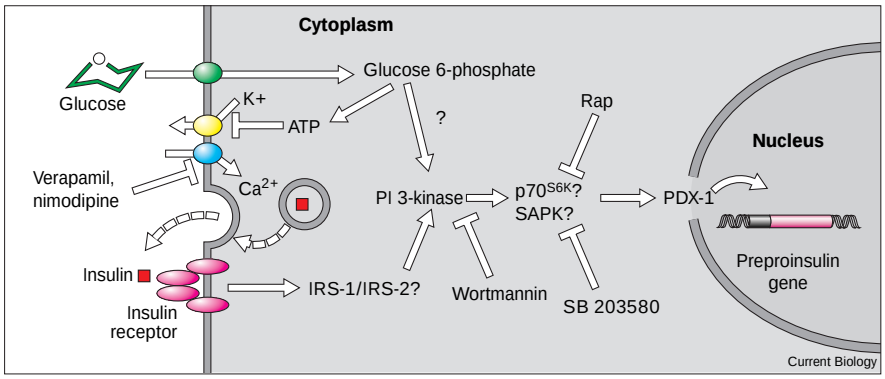
Kulkarni *et al.* [1] have addressed the first of these points in intact mice by ablating insulin receptors — members of the receptor tyrosine kinase family — specifically in islet β cells. To do this, they first targeted expression of the Cre recombinase enzyme specifically to islet β cells using the rat insulin promoter. Cre recombinase is capable of removing transgenes inserted between specific sequences, known as *loxP* sites. The resulting animals were bred with transgenic mice in which exon 4 of the insulin receptor gene was flanked with *loxP* sites. In the progeny carrying both the Cre and *loxP* constructs, excision of exon 4 in β cells resulted in the production — in these cells only — of truncated, non-functional insulin receptors. Crucially, pancreatic islet development was normal in these 'βIRKO' animals, although a decline in pancreatic insulin content and islet size was observed with age.

The βIRKO animals are not overtly diabetic, and display only mild hyperinsulinaemia. But in contrast to mice lacking muscle insulin receptors [9], they display reduced glucose tolerance and a drastically reduced 'first phase' of insulin secretion — classical features of NIDDM in humans. This may indicate that there are effects of insulin, not only on the preproinsulin gene, but on other β cell genes as well. These may include genes encoding key 'glucose sensing' enzymes, such as glucokinase, the glucose transport GLUT2 and lactate dehydrogenase [10], so that a defect in the regulation would lead to abnormal glucose metabolism. Any such effects on the expression of these genes may be a consequence of transcription factor dysregulation, as has been observed in certain forms of the disorder known as 'maturity-onset diabetes of the young' [11]. *In vitro* studies with islets from βIRKO animals will be necessary to determine whether this is the case.

A direct effect on preproinsulin gene expression?

Using reporter constructs encoding the green fluorescent protein (GFP) [12], Leibiger *et al.* [2] showed that short incubations of isolated rat islets or HIT-T15 insulinoma cells with physiological concentrations of insulin activated transcription directed by the insulin promoter. Other studies have examined the effects of insulin on the transcription factor known as 'pancreatic duodenum *Xenopus* homeodomain factor-1' (PDX-1) [13] — variously known

Figure 1



The possible interplay between the action of glucose (top left) and secreted insulin (red squares) on preproinsulin gene expression in the islet β cell. (See text for details.)

by the alternative names IPF-1, IDX-1, STF-1 or IUF1 — a key player in the regulation of insulin promoter activity [14]. MacFarlane *et al.* [15] have recently found that PDX-1 binding to cognate DNA is stimulated in human islets by added insulin, apparently mimicking the effect of glucose. Whether insulin, like glucose, causes translocation of PDX-1 to the nucleoplasm [14,15] is as yet untested.

One further molecular target of insulin may be the phosphoprotein known as 'phosphorylated heat- and acid-stable protein regulated by insulin' (PHAS-I), a regulator of translation initiation. Xu *et al.* [3] have recently shown that the phosphorylation state of this protein in pancreatic islets is increased by exposure to glucose or exogenous insulin, an action predicted to enhance the translation of preproinsulin mRNA.

Are there parallel effects of glucose on insulin secretion and preproinsulin gene expression?

In contrast to recent findings described above, which have provided compelling evidence for a role of secreted insulin in the regulation of gene expression, studies of insulin secretion have provided a less clear-cut picture. Indeed, the intracellular signalling pathways leading to insulin exocytosis, on the one hand, and to enhanced gene expression, on the other, have long been thought to be distinct. Work more than twenty-five years ago [16] showed that insulin biosynthesis, as measured through incorporation of radiolabelled leucine, displays a lower threshold than secretion for glucose activation, and is unaffected by removal of extracellular Ca^{2+} ions. The role of Ca^{2+} is particularly important in this context, as blockade of Ca^{2+} influx inhibits glucose-induced insulin secretion under most conditions by at least 95%. Furthermore, increases in intracellular [Ca^{2+}], and activation of secretion by the oral antihyperglycaemic agent tolbutamide, have no effect on insulin biosynthesis measured this way [6].

These measurements chiefly reflect preproinsulin mRNA translation, which would therefore appear not to be regulated by secreted insulin. But this does not exclude a role for insulin in the activation of preproinsulin gene transcription. Here, the role of intracellular Ca^{2+} concentration — and therefore potentially of secreted insulin — is much more controversial. Thus, in a recent study using GFP reporter genes imported into islets from adult rats using adenovirus vectors [17], no effect of pharmacological Ca^{2+} channel blockade with verapamil was observed on insulin promoter activity. Similarly, others observed only small effects of inhibiting Ca^{2+} influx on insulin promoter activity in HIT-T15 insulinoma [18] and INS-1 cells (G.A.R. and H.J. Kennedy, unpublished data). Clear effects were, however, seen in transfected foetal islets [19] and in β-TC3 insulinoma cells [20].

Most recently, Leibiger *et al.* [2] observed that Ca^{2+} channel blockade with nimodipine reversed glucose-induced increases in preproinsulin gene promoter activity and mRNA levels in islets and HIT-T15 cells. Furthermore, the effects of glucose (and of insulin) could be mimicked by increasing Ca^{2+} influx. By contrast, Docherty's group has found that PDX-1 binding to DNA is not affected by Ca^{2+} removal, nor mimicked by Ca^{2+} influx (K. Docherty, personal communication). Similarly, inhibition of phosphatidylinositol 3-kinase (PI 3-kinase) with wortmannin blocked glucose-activated DNA binding by PDX-1 completely at wortmannin concentrations that do not affect glucose-stimulated insulin release. Thus there is much evidence, at least *in vitro*, to cast doubt on a simple scheme in which secreted insulin is alone responsible for activation of the preproinsulin gene by glucose.

An acute effect of insulin on its own secretion?

The question of whether insulin has an acute effect on its own secretion has been the most studied aspect of insulin's influence on pancreatic islets. Here again, the picture is complicated by the number and type of preparations used. Most early studies showed a clear inhibitory effect on secretion of added insulin, in static incubation of rat islets [21], in the perfused pancreas [22] or after intravenous

insulin injection and maintenance of euglycaemia in man [23]. Other data, however, have suggested there is no acute effect of insulin, or an effect that requires pancreatic innervation, on insulin secretion [24]. Using amperometry to detect insulin secreted from single β cells, Aspinwall *et al.* [4] have recently observed that insulin directly and rapidly activates its own secretion.

Conclusions

Taken together, the above data suggest a scheme for the regulation of preproinsulin gene expression that is illustrated in Figure 1. What intracellular signalling pathways may be involved downstream of the insulin receptor in β cells? At this stage, the pharmacological data are still controversial. It seems likely that glucose and insulin may act through separate pathways initially, perhaps converging downstream on a common pathway. The reported effects of wortmannin, sensitivity to rapamycin and a lack of effect of dominant-negative forms of protein kinase B [2] indicate the action of a signalling pathway from the insulin receptor that involves PI 3-kinase, protein kinase p70, S6 kinase and a stress-activated protein kinase (SAPK) [15]. Importantly, such a scheme could explain the observed β cell defects observed in mutant mice lacking insulin receptor substrate-2 (IRS-2), a known downstream target of the insulin receptor [25]. It will be important to determine how far the effects of insulin and glucose are additive, as it may be that secreted insulin acts largely to potentiate the effects of submaximal glucose concentrations. As a result, defective action of insulin on the β cell — 'insulin resistance' — may be an important contributor to the pathology of NIDDM.

Acknowledgements

Work in the author's laboratory is supported by grants from the Medical Research Council (UK), the Wellcome Trust, The British Diabetic Association, The Biotechnology and Biological Sciences Research Council, the European Community and The Royal Society. I thank Isabelle Leclerc for careful revision of the text.

References

1. Kulkarni RN, Bruning JC, Winnay JN, Postic C, Magnuson MA, Kahn CR: Tissue-specific knockout of the insulin receptor in pancreatic beta cells creates an insulin secretory defect similar to that in type 2 diabetes. *Cell* 1999, 96:329-339.
2. Leibiger IB, Leibiger B, Moede T, Berggren PO: Exocytosis of insulin promotes insulin gene transcription via the insulin receptor PI-3 kinase p70 s6 kinase and CaM kinase pathways. *Mol Cell* 1998, 1:933-938.
3. Xu G, Marshall CA, Lin TA, Kwon G, Munivenkatappa RB, Hill JR, Lawrence JC, McDaniel ML: 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* 1998, 273:4485-4491.
4. Aspinwall CA, Lakey JR, Kennedy RT: Insulin-stimulated insulin secretion in single pancreatic beta cells. *J Biol Chem* 1999, 274:6360-6365.
5. Nielsen DA, Welsh M, Casadaban MJ, Steiner DF: Control of insulin gene expression in pancreatic β-cells and in an insulin-producing cell line, RIN-5F cells I effects of glucose and cyclic AMP on the transcription of insulin mRNA. *J Biol Chem* 1985, 260:13585-13589.
6. Ashcroft SJ, Bunce J, Lowry M, Hansen SE, Hedeskov CJ: The effect of sugars on (pro)insulin biosynthesis. *Biochem J* 1978, 174:517-526.
7. Lang JC: Molecular mechanisms and regulation of insulin exocytosis as a paradigm of endocrine secretion. *Eur J Biochem* 1999, 259:3-17.
8. Bailyes EM, Guest PC, Hutton JC: Insulin synthesis. In *Insulin, Molecular Biology to Pathology*. Edited by Ashcroft FM, Ashcroft SJH. Oxford, New York, Toronto: Oxford University Press; 1992:64-92.
9. Bruning JC, Michael MD, Winnay JN, Hayashi T, Horsch D, Accili D, Goodyear LJ, Kahn CR: A muscle-specific insulin receptor knockout exhibits features of the metabolic syndrome of NIDDM without altering glucose tolerance. *Mol Cell* 1998, 2:559-569.
10. Sekine N, Cirulli V, Regazzi R, Brown LJ, Gine E, Tamarit-Rodriguez J, Girotti M, Marie S, MacDonald MJ, Wollheim CB, Rutter GA: Low lactate dehydrogenase and high mitochondrial glycerol phosphate dehydrogease in pancreatic β-cell. Potential role in nutrient sensing. *J Biol Chem* 1994, 269:4895-4902.
11. Yamagata K, Furuta H, Oda N, Kaisaki PJ, Menzel S, Cox NJ, Fajans SS, Signorini S, Stoffel M, Bell GI: Mutations in the hepatocyte nuclear factor-4alpha gene in maturity-onset diabetes of the young (MODY1). *Nature* 1996, 384:458-460.
12. Rutter GA, Kennedy HJ, Wood CD, White MRH, Tavare JM: Quantitative real-time imaging of gene expression in single cells using multiple luciferase reporters. *Chem Biol* 1998, 5:R285-R290.
13. Ohlsson H, Karlsson K, Edlund T: IPF-1, a homeodomain-containing transactivator of the insulin gene. *EMBO J* 1993, 12:4251-4259.
14. Rafiq I, Kennedy H, Rutter GA: Glucose-dependent translocation of insulin promoter factor-1 (IPF-1) between the nuclear periphery and the nucleoplasm of single MIN6 β-cells. *J Biol Chem* 1998, 273:23241-23247.
15. MacFarlane WM, McKinnon CM, Felton-Edkins ZA, Cragg H, James RL, Docherty K: Glucose stimulates translocation of the homeodomain transcription factor PDX1 from the cytoplasm to the nucleus in pancreatic beta-cells. *J Biol Chem* 1999, 274:1011-1016.
16. Pipeleers DG, Marical M, Malaisee WJ: The stimulus-secretion coupling of glucose-induced insulin release. XIV. Glucose regulation of insular biosynthetic activity. *Endocrinology* 1973, 93:1001-1011.
17. deVargas LM, Sobolewski J, Siegel R, Moss LG: Individual beta cells within the intact islet differentially respond to glucose. *J Biol Chem* 1997, 272:26573-26577.
18. Goodison S, Kenna S, Ashcroft SJH: Control of insulin gene expression by glucose. *Biochem J* 1992, 285:563-568.
19. German MS, Moss LG, Rutter WJ: Regulation of insulin gene expression by glucose and calcium in transfected primary islet cultures. *J Biol Chem* 1990, 265:22063-22066.
20. Efrat S, Surana M, Fleischer N: Glucose induces insulin gene transcription in a murine pancreatic beta cell line. *J Biol Chem* 1991, 266:11141-11143.
21. Malaisse WJ, Malaisse-Lagae F, Lacy PE, Wright PH: Insulin secretion by isolated islets in presence of glucose, insulin and anti-insulin serum. *Proc Soc Exp Biol Med* 1967, 124:497-500.
22. Iversen J, Miles DW: Evidence for a feedback inhibition of insulin on insulin secretion in the isolated, perfused canine pancreas. *Diabetes* 1971, 20:1-9.
23. Elahi D, Nagulesparan M, Hershcopf RJ, Muller DC, Tobin JD, Blix PM, Rubenstein AH, Unger RH, Andres R: Feedback inhibition of insulin secretion by insulin: relation to the hyperinsulinemia of obesity. *N Engl J Med* 1982, 306:1196-1202.
24. Stagner J, Samols E, Polonsky K, Pugh W: Lack of direct inhibition of insulin secretion by exogenous insulin in the canine pancreas. *J Clin Invest* 1986, 78:1193-1198.
25. Withers DJ, Gutierrez JS, Towery H, Burks DJ, Ren JM, Previs S, Zhang Y, Bernal D, Pons S, Shulman GI, *et al.*: Disruption of IRS-2 causes type 2 diabetes in mice. *Nature* 1998, 391:900-904.