Insulin receptor activation inhibits insulin secretion from human islets of Langerhans

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Abstract There is no consensus on the role of insulin secreted from pancreatic β-cells in regulating its own secretion, either in rodent islets or in human islets. We have now investigated whether there is an autocrine signalling role for insulin in human islets by determining insulin receptor expression and assessing the effects of insulin receptor activation using a non-peptidyl insulin mimetic termed L-783,281. Human insulin receptor mRNA was detected by PCR amplification of human islet cDNA, and translation of the message in human islets was confirmed by Western blotting. Perifusion experiments revealed that both glucose-stimulated and basal insulin secretion were significantly inhibited following human islet insulin receptor activation with L-783,281, and that signalling through phosphatidylinositol 3-kinase (PI 3-kinase) was responsible, at least in part, for this inhibitory effect. These studies indicate that human islets express insulin receptors and that they are functionally coupled to a PI 3-kinase-dependent inhibition of insulin secretion.

Key words: Human islet of Langerhans; Insulin secretion; Autocrine; Perifusion

1. Introduction

The regulated release of insulin from pancreatic β-cells is pivotal for the maintenance of serum glucose concentrations within the physiological range. Glucose itself is the major physiological stimulus for insulin secretion, but a number of hormones and neurotransmitters can act at β-cells to modulate the secretory process [1]. It has long been known that glucagon released from pancreatic α-cells potentiates glucose-stimulated insulin secretion [2], while the δ-cell hormone somatostatin inhibits insulin secretion [3]. However, there is considerable uncertainty about the autocrine effects of insulin on insulin secretion from β-cells. In other cell types it is well-established that insulin binds to the heterotetrameric insulin receptor, stimulating its intrinsic tyrosine kinase activity, leading to autophosphorylation of the receptor β subunits and tyrosine phosphorylation of intracellular proteins known as insulin receptor substrates (IRS). The phosphorylated IRS proteins act as docking proteins for SH2-containing effector proteins that mediate the signalling events initiated by insulin [4].

Radioligand binding studies have suggested that purified islet β-cells do not express high affinity insulin receptors [5], while more recent studies using single cell polymerase chain reaction (PCR) have indicated that rat β-cells co-express insulin, insulin receptor and IRS-1 mRNAs [6]. The lack of consensus in this area extends to the effects of insulin on insulin secretion. There have been reports that insulin does not affect insulin secretion [7–9], that it inhibits [10–13], or that it stimulates [14–16] insulin secretion. Some of the confusion arising from these studies may have resulted from the measurement of insulin levels by radioimmunoassay following exposure to insulin in vitro, making it difficult to distinguish between secretion of endogenous hormone and the contribution made by the exogenous insulin. Further discrepancies in the literature may also be caused by comparing in vivo studies in humans with in vitro experiments primarily using islets or β-cell lines derived from rodents. Other potentially confounding factors include the use of non-physiological concentrations of insulin; measurement of insulin release in static incubations where potentially inhibitory products could accumulate; in vivo studies where the responses do not necessarily reflect direct effects of insulin on the islets; and indirect estimates of β-cell secretory activity using microelectrodes. Despite the lack of consensus regarding the effects of insulin on the exocytotic release of insulin from β-cells, studies using rodent islets and HIT-T15 cells have provided very good evidence that insulin stimulates insulin gene transcription through phosphatidylinositol 3-kinase (PI 3-kinase) Ia/p70 S6 kinase and CaM kinase pathways [17,18], and that it regulates glucokinase gene transcription through PI 3-kinase II/protein kinase B pathways [18]. The capacity of insulin to exert biological effects on gene transcription in islets and a β-cell line strongly supports the presence of insulin receptors and downstream signalling elements by β-cells.

Many of the experimental difficulties associated with identifying the effect of insulin on insulin secretion can be circumvented by selectively activating insulin receptors with an insulin-mimetic which is without insulin immunoreactivity. A non-peptidyl fungal metabolite, termed L-783,281, has been shown to mimic many of insulin's effects in vitro and in vivo through activation of the insulin receptor β subunit tyrosine kinase activity [19], and this agent has been reported to stimulate insulin gene expression in mouse β-cells [16]. We have now used L-783,281 to establish the consequences of short-term activation of insulin receptors on dynamic insulin secretion from human islets of Langerhans.

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Abbreviations: IRS, insulin receptor substrate; PI 3-kinase, phosphatidylinositol 3-kinase

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2. Materials and methods

2.1. Human islet isolation

Human islets were provided by Dr Guo Cai Huang of the Dixon's Human Islet Project (King's College Hospital, London, UK). Briefly, pancreata were retrieved, with permission, from non-diabetic heart-beating cadaver organ donors, and islets were isolated under aseptic conditions according to the method of London and colleagues [20]. Islets were used after maintenance overnight in CMRL medium supplemented with 15% foetal calf serum, 100 U/ml penicillin and 100 μg/ml streptomycin. All tissue culture reagents were obtained from Gibco (Paisley, UK). Electron micrographs indicated that hand-picked human islets were >90% pure.

2.2. PCR

Messenger RNA was isolated from hand-picked human islets using a Dynabeads Oligo$(dT)_{25}$ kit according to the manufacturer's instructions (Dynal, Merseyside, UK). Oligo$(dT)_{18}$ (1 μg) and random 10-mers (1 μg) were added to islet mRNA (10 μl) and the mixture was heated (70°C, 5 min) to remove secondary RNA structure, then cooled on ice. Dithiothreitol (10 mM), dATP, dCTP, dTTP and dGTP (all 0.5 mM), recombinant ribonuclease inhibitor (80 U, RNasin), MMLV-RT (200 U) and molecular biology grade water were added to give a final volume of 20 μl, and the mixture was incubated at 42°C for 50 min. MMLV-RT was inactivated by heating at 70°C for 15 min. The cDNA was amplified by 30 cycles of PCR using primers that detected human insulin receptor (forward: 5′-CAG CGA GAA ACT GCA TGG T-3′; reverse: 5′-CAT TGG ACA TGG TAG AGT CG-3′). The predicted size of the human insulin receptor PCR product was 770 bp. PCR reactions were also performed using MIN6 β-cells and mouse liver, using primers that detected the mouse insulin receptor (forward: 5′-ATC TGG ATC CCC CTG ATA ACT GTC-3′; reverse: 5′-ATG TGG GTG TAG GGG ATG TGT TCA-3′). The predicted size of the mouse insulin receptor product was 331 bp. PCR products amplified by the insulin receptor primers were separated by agarose gel electrophoresis (2% w/v) and visualised by staining with ethidium bromide (0.5 μg/ml). The PCR products were excised from the gels and their identities were confirmed by sequencing using standard fluorescent chain-terminator methods.

2.3. Western blot analysis

Human islets were hand-picked from the islet digest provided by Dr Huang, using a finely drawn capillary glass pipette and transferred to polyacrylamide gel electrophoresis sample buffer [21]. Human islet proteins were fractionated by electrophoresis on 10% polyacrylamide Bis-Tris gels (Invitrogen, Groningen, The Netherlands) and transferred to polyvinylidene fluoride membranes using the Novex Xcell II blotting apparatus (Invitrogen). Insulin receptor expression was identified by immunoprobing membranes with a rabbit polyclonal antibody raised against the insulin receptor α subunit (Autogen Bioclear, Wiltshire, UK) and a horseradish peroxidase-linked goat anti-rabbit secondary antibody (Pierce, Rockford, IL, USA). Immunoreactive proteins were detected by ECL™ (Amersham International plc, Bucks, UK).

2.4. Insulin secretion

Human islets were transferred to Swinnex chambers (Millipore, Bedford, MA, USA) containing 1 μm pore-size nylon filters and perifused at a flow rate of 0.5 ml/min at 37°C in a temperature-controlled environment. Islets were initially perifused with a physiological buffer [22] containing 2 mM glucose for 1 h and insulin secreted from the islets into the perifusate during this period was discarded. Islets were then perifused with buffers containing 2 or 20 mM glucose, either in the absence or in the presence of 10 μM L-783,281 (Merck Research Laboratories, Rahway, NJ, USA). L-783,281 was stored as a 20 mM stock in dimethyl sulfoxide (DMSO) at −20°C. For use in experiments it was initially diluted to 5 mM in DMSO, then further diluted to 10 μM in protein-free aqueous buffer. In some experiments buffers were supplemented with 20 μM LY 294002 (Calbiochem, Nottingham, UK), a PI 3-kinase inhibitor. Perifusate fractions were collected every 2 min and insulin content was determined by radioimmunoassay [21]. The effects of DMSO alone were examined in static incubations where groups of three hand-picked human islets were incubated for 30 min at 37°C at 2 mM or 20 mM glucose in the absence or presence of 0.2% (v/v) DMSO, and insulin content of the supernatant was assessed by radioimmunoassay.

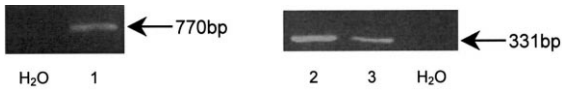

Fig. 1. Amplification of insulin receptor cDNA in human islets (lane 1), mouse liver (lane 2) and MIN6 β-cells (lane 3). PCR products were separated on 2% agarose gels. No products were obtained when water rather than cDNA was included in the PCR reactions.

3. Results

3.1. Human islets express insulin receptor mRNA

Fig. 1 shows images of ethidium bromide-stained agarose gels in which insulin receptor cDNA had been amplified from hand-picked human islets (left) and mouse liver and MIN6 cells (right). Products of the appropriate sizes were obtained and their identities were confirmed by sequencing using fluorescent chain-terminator methods.

3.2. Human islet insulin receptor mRNA is translated into protein

Translation of insulin receptor mRNA into protein was confirmed by Western blotting using an antibody raised against the extracellular α subunit of the insulin receptor. It can be seen from Fig. 2 that both human islets and mouse skeletal muscle contained a 130 kDa immunoreactive protein, consistent with the molecular mass of the insulin receptor α subunit and indicative of the presence of the insulin receptor complex in human islet cells as well as in mouse skeletal muscle.

3.3. Activation of human islet insulin receptors inhibits insulin secretion

Perifusion experiments with human islets isolated from two separate donors indicated that insulin secretion was significantly stimulated upon raising the glucose concentration from 2 mM to 20 mM, as expected (Fig. 3, upper panel). Addition of the insulin mimetic, L-783,281, at 10 μM resulted in a significant ($P < 0.05$) inhibition of glucose-induced insulin secretion, such that the secretory response approached basal levels of insulin secretion (Fig. 3, upper panel). The effects of activation of human islet insulin receptors with L-783,281 at 2 mM glucose were also examined. It can be seen from Fig. 3 (lower panel) that 10 μM L-783,281 elicited a rapid inhibition of basal insulin secretion that was partially reversible when the perifusion medium was returned to 2 mM glucose alone. The inhibitory effects of L-783,281 were mediated, at least in part, through a PI 3-kinase-sensitive pathway, since they were reduced in the presence of the PI 3-kinase inhibitor LY 294002 (2 mM glucose+10 μM L-783,281: 53 ± 2.0% basal

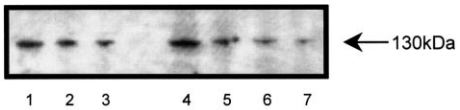

Fig. 2. Insulin receptor α subunit immunoreactivity in mouse skeletal muscle (lanes 1–3) and hand-picked human islets (lanes 4–7). 20, 10 and 5 μg protein was loaded on to lanes 1–3, and protein from 40, 20, 10 and five hand-picked human islets was loaded on to lanes 4–7.

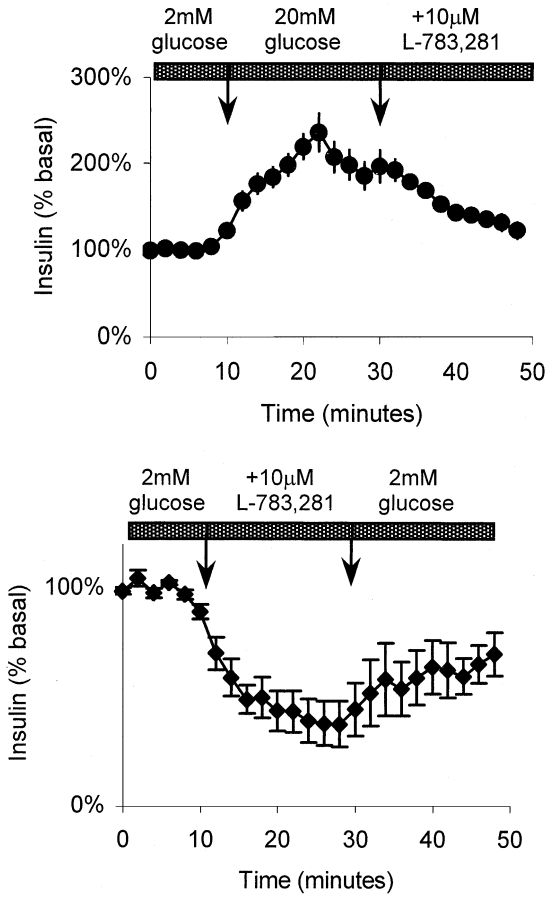

Fig. 3. Inhibition of glucose-stimulated (upper panel) and basal (lower panel) insulin secretion from perifused human islets by L-783,281. Data show means ± S.E.M. of eight separate perifusion chambers using islets isolated from two donors. Where error bars are not shown they are smaller than the size of the symbol.

secretion; +20 μM LY 294002: 107 ± 9.4% basal, $n = 3$, $P < 0.01$). However, the capacity of 20 μM LY 294002 alone to stimulate secretion at 2 mM glucose (144 ± 9.8% basal secretion) makes it difficult to fully define the role of PI 3-kinase in the inhibition of secretion by L783,281.

Inhibition of insulin secretion by L783,281 could not be attributed to vehicle since 0.2% DMSO did not have any significant effect on basal nor glucose-stimulated insulin secretion (2 mM glucose: 0.35 ± 0.02 ng/islet/30 min; 2 mM glucose+DMSO: 0.34 ± 0.02; 20 mM glucose: 1.03 ± 0.06; 20 mM glucose+DMSO: 1.08 ± 0.08; $n = 7$–8; $P < 0.0001$, 2 mM vs 20 mM glucose; $P > 0.2$ for the effect of DMSO).

4. Discussion

There is very little information available about the regulation of insulin secretion from human islets as a result of the scarcity of human islet tissue available for experimentation, and to date there are no publications on the expression and effects of activation of human islet insulin receptors in vitro.

The current study has shown that isolated human islets express mRNA for the human insulin receptor and that this message is translated into protein since the 130 kDa insulin receptor α subunit was also detected by Western blotting. It is not clear from our experiments which islet cell type expresses the insulin receptor, but given that human islets contain at least 50% β-cells [23] and that isolated α-cells do not bind insulin [24], it seems likely that some, if not all, of the insulin receptor immunoreactivity on human islets can be attributed to β-cells. In addition, since insulin receptor mRNA could be detected in a mouse β-cell line (MIN6) by PCR, it seems likely that insulin receptors are also expressed on human β-cells.

The insulin receptors on human islets are functionally coupled to insulin secretion, as evidenced by the effect of L-783,281, an insulin mimetic, on insulin secretion from perifused human islets. Our data support a role for insulin provoking a negative feedback effect on β-cells to inhibit both sustained glucose-stimulated insulin secretion and unstimulated insulin secretion. Thus, the inhibition of glucose-induced insulin release evoked by L-783,281 is consistent with several earlier studies [10–13], but directly contradicts others [14,15]. The reasons for the lack of concordance between the results of the present study and those of Aspinwall and colleagues [14,15] are not clear, but may reflect species differences (human versus mouse islets) or differences in the method used to measure insulin secretion (radioimmunoassay versus amperometry). The inhibition of secretion seen in the current study is consistent with the recent observation that insulin hyperpolarises mouse β-cells through activation of ATP-sensitive K^+ channels [25].

Our observation that activation of islet insulin receptors resulted in a profound inhibition of basal insulin secretion was somewhat unusual as modulators of β-cell function rarely inhibit unstimulated insulin release. However, the inhibitory profile at 2 mM glucose obtained in response to L-783,281 in the current studies is reminiscent of that seen in response to elevated extracellular Ca^{2+} levels in human islets [26], and it is clear that insulin secretion obtained in the absence of stimulatory concentrations of glucose is a regulated process, rather than just leakage. The inhibitory effect of L-783,281 on basal secretion was also surprising since insulin might be expected to inhibit insulin secretion by a negative feedback route only after its secretion in response to β-cell stimulation. However, the current data are indicative of a role for insulin released from unstimulated β-cells in maintaining the β-cells in a basal state, potentially preventing dysregulated insulin secretory output and fully support the proposal of Khan and colleagues that insulin has a physiological autocrine role as a negative feedback signal in β-cells [25]. These observations are also consistent with those of a previous study in which selective inactivation of the β-cell insulin receptor gene in mice was associated with significant increases in their fasting insulin levels [27].

Previous studies using PI 3-kinase inhibitors have demonstrated the involvement of this enzyme in transducing insulin-mediated signalling in rodent β-cells [17,18,25,28], and it has been reported that PI 3-kinase is active in β-cells under non-stimulatory conditions [29]. The reduced inhibitory capacity of L-783,281 in the presence of LY 294002, a PI 3-kinase inhibitor, suggests a key role for PI 3-kinase in transducing the L-783,281-mediated inhibition of insulin secretion from human islets. The raised basal secretion in the presence of LY 294002 is also consistent with a tonic inhibition of insulin secretion through a PI 3-kinase-sensitive pathway, and confirms previously published data in the MIN6 insulin-secreting cell line [30].

In conclusion, this study has shown that human islets, like

rodent islets and β-cell lines, express functionally active insulin receptors and that insulin secreted from human pancreatic β-cells acts in an autocrine manner at insulin receptors to negatively regulate its own secretion. Such a negative feedback mechanism may play a role in the biphasic profile of insulin secretion, as has been proposed by other investigators [11], but our study suggests that it is also important in regulating the basal insulin secretory activity of β-cells. Our demonstration of autocrine regulation of β-cell function in primary human islets raises the intriguing possibility that defects in this regulatory system are involved in the development of pathologies associated with dysfunctional insulin secretion, such as Type 2 diabetes [31] or persistent hyperinsulinaemic hypoglycaemia of infancy [32].

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References

[1] Ahrén, B. (1999) Adv. Mol. Cell Biol. 29, 175–197.
[2] Pipeleers, D.G., Schuit, F.C., Van Schravendijk, C.F.H. and Van de Winkel, M. (1985) Endocrinology 117, 824–833.
[3] Taminato, T., Seino, Y., Goto, Y. and Imura, H. (1975) Biochem. Biophys. Res. Commun. 66, 928–934.
[4] White, M.F. (1997) Diabetologia 40, S2–S17.
[5] Pipeleers, D. (1984) Experientia 40, 1114–1129.
[6] Harbeck, M.C., Louie, D.C., Howland, J., Wolf, B.A. and Rothenberg, P.L. (1996) Diabetes 45, 711–717.
[7] Schatz, H. and Pfeiffer, E.F. (1977) J. Endocrinol. 74, 243–249.
[8] Marincola, F., Frank, W., Clark, W., Douglas, M. and Merrell, R. (1983) Diabetes 32, 1162–1167.
[9] Rothenberg, P.L., Willison, D., Simon, J. and Wolf, B.A. (1995) Diabetes 44, 802–809.
[10] Ammon, H.P.T. and Verspohl, E. (1976) Endocrinology 99, 1469–1476.
[11] Pace, C.S., Matschinsky, F.M., Lacy, P.E. and Conant, S. (1977) Biochim. Biophys. Acta 497, 408–414.
[12] Draznin, B., Goodman, M., Leitner, J.W. and Sussman, K.E. (1986) Endocrinology 118, 1054–1058.
[13] Van Schravendijk, C.F.H., Heylen, L., Van den Brande, J.L. and Pipeleers, D.G. (1990) Diabetologia 33, 649–653.
[14] Aspinwall, C.A., Lakey, J.R.T. and Kennedy, R.T. (1999) J. Biol. Chem. 274, 6360–6365.
[15] Aspinwall, C.A., Qian, W.J., Roper, M.G., Kulkarni, R., Kahn, C.R. and Kennedy, R.T. (2000) J. Biol. Chem. 275, 22331–22338.
[16] Xu, G.G., Gao, Z.Y., Borge, P.D., Jegier, P.A., Young, R.A. and Wolf, B.A. (2000) Biochemistry 39, 14912–14919.
[17] Leibiger, I.B., Leibiger, B., Moede, T. and Berggren, P.-O. (1998) Mol. Cell 1, 933–938.
[18] Leibiger, B., Leibiger, I.B., Moede, T., Kemper, S., Kulkarni, R., Kahn, C.R., Moitoso de Vargas, L. and Berggren, P-O. (2001) Mol. Cell 7, 559–570.
[19] Zhang, B., Salituro, G., Szalkowski, D., Li, Z., Zhang, Y., Royo, I., Vilella, D., Diez, M.T., Pelaez, F., Ruby, C., Kendall, R.L., Mao, X., Griffin, P., Calaycay, J., Zierath, J.R., Heck, J.V., Smith, R.G. and Moller, D.E. (1999) Science 284, 974–977.
[20] London, N.J.M., James, R.F.L. and Bell, P.R.F. (1992) in: Pancreatic Islet Cell Transplantation (Ricordi, C., Ed.), pp. 113–123, R.G. Landes, Austin, TX.
[21] Jones, P.M., Salmon, D.M.W. and Howell, S.L. (1988) Biochem. J. 254, 397–403.
[22] Gey, G.O. and Gey, M.K. (1936) Am. J. Cancer 27, 45–76.
[23] Bishop, A.E. and Polok, J. (1997) in: Textbook of Diabetes, Vol. 1 (Pickup, J.C. and Williams, G., Eds.), pp. 6.1–6.16, Blackwell Science Ltd, Oxford.
[24] Van Schravendijk, C.F.H., Foriers, A., Hooghe-Peters, E.L., Rogiers, V., de Meyts, P. and Pipeleers, D.G. (1985) Endocrinology 117, 841–848.
[25] Khan, F.A., Goforth, P.B., Zhang, M. and Satin, L.S. (2001) Diabetes 50, 2192–2198.
[26] Squires, P.E., Harris, T.E., Persaud, S.J., Curtis, S.B., Buchan, A.M.J. and Jones, P.M. (2000) Diabetes 49, 409–417.
[27] Kulkarni, R.N., Brüning, J.C., Winnay, J.N., Postic, C., Magnuson, M.A. and Kahn, C.R. (1999) Cell 96, 329–339.
[28] Xu, G., Marshall, C.A., Lin, T.-A., Kwon, G., Munivenkatappa, R.B., Hill, J.R., Lawrence, J.C. and McDaniel, M.L. (1998) J. Biol. Chem. 273, 4485–4491.
[29] Alter, C.A. and Wolf, B.A. (1995) Biochem. Biophys. Res. Commun. 208, 190–197.
[30] Hagiwara, S., Sakurai, T., Tashiro, F., Hashimoto, Y., Matsuda, Y., Nonomura, Y. and Miyazaki, J-I. (1995) Biochem. Biophys. Res. Commun. 214, 51–59.
[31] Matthews, D.R. and Clark, A. (1997) in: Textbook of Diabetes, Vol. 1 (Pickup, J.C. and Williams, G., Eds.), pp. 21.1–21.16, Blackwell Science Ltd, Oxford.
[32] Aynsley-Green, A., Polak, J.M., Bloom, S.R., Gough, M.H., Keeling, J., Ashcroft, S.J.H., Turner, R.C. and Baum, J.D. (1981) Arch. Dis. Child. 56, 496–508.