Direct effects of rapid-acting insulin analogues on insulin signaling in human pancreatic islets in vitro

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Abstract

Aims. – Studies suggest that insulin-signaling molecules are present in the pancreatic islets. For this reason, the effects of insulin glulisine, insulin aspart and regular human insulin (RHI) on the function and molecular features of isolated human pancreatic islets were investigated.

Methods. – Human pancreatic islets were prepared by collagenase digestion and density-gradient purification of pancreata from multiple organ donors. Islets were then cultured for 48 h in the presence of 5.5 (normal) or 22.2 (high) mmol/L of glucose with and without glulisine, aspart and RHI (10 or 100 nmol/L). Functional (glucose-stimulated insulin secretion) and molecular (quantitative RT-PCR and immunoblot) studies were performed at the end of the different incubation conditions.

Results. – Glucose-stimulated insulin secretion was blunted in islets cultured in 22.2 mmol/L of glucose, with no significant effects from the exogenous added insulins. In islets maintained at 5.5 mmol/L of glucose, insulin receptor (IR) expression was reduced by low RHI, while phosphatidylinositol-3 kinase p110-alpha (PI3K) was enhanced by both concentrations of glulisine and aspart, and by high RHI. In islets preexposed to high glucose, IR expression was increased by both concentrations of aspart and RHI, but not by glulisine. Glulisine at high concentration significantly increased PI3K expression. Glulisine and RHI significantly increased IRS-2 phosphorylation compared with control and aspart (P < 0.05).

Conclusion. – Insulin analogues have differential effects on the expression of insulin-signaling molecules in human pancreatic islets that are also dependent on the degree of glucose exposure.

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Keywords: Insulin analogue; Glulisine; β-cell; IRS-2

Résumé


Objectifs. – Des études suggèrent que les molécules impliquées dans la voie de signalisation de l’insuline sont présentes au niveau des îlots pancréatiques. Nous avons analysé les effets de l’insuline glulisine (glulisine), de l’insuline asparte (asparte) et de l’insuline rapide humaine (IRH) sur la fonction et certains aspects moléculaires d’îlots pancréatiques humains isolés.

Méthodes. – Les îlots pancréatiques humains ont été préparés par digestion enzymatique par la collagénase et purifiés par gradient de densité à partir de pancréas de donneurs multi-organes. Les îlots ont ensuite été cultivés pendant 48 heures en présence de glucose à 5,5 mmol/L (concentration normale) ou 22,2 mmol/L (concentration élevée), avec ou sans glulisine, asparte ou IRH (10 ou 100 nmol/L). Des études fonctionnelles (sécrétion d’insuline induite par le glucose) et moléculaires (RT-PCR quantitative et immunotransfert) ont été réalisées à la fin des périodes d’incubation dans ces différentes conditions.

Résultats. – La sécrétion d’insuline induite par le glucose est réduite dans les îlots cultivés en présence de 22,2 mmol/L sans aucun effet significatif de l’addition d’une insuline exogène. Dans les îlots maintenus à 5,5 mmol/L de glucose, l’expression du récepteur à
Diabetes is a systemic disorder that arises when insulin release from pancreatic β cells fails to maintain blood glucose concentrations within the normal range. In type 2 diabetes, the most common form of diabetes, pancreatic β-cell failure is exacerbated by preexisting insulin resistance. Recent studies suggest that many proteins of the insulin-signaling pathway are present and play a physiological role not only in classical insulin signaling, including IRS-1 and -2, but also in the β cells of the pancreatic islets [1]. In fact, tissue-specific knockout of the insulin receptor in pancreatic β cells leads to altered glucose-sensing and glucose intolerance in adult mice [2]. In addition, studies in cell lines and rodents have shown that insulin receptor substrate (IRS)-1-deficient β cells display a reduced response to glucose and arginine, and that the absence of IRS-2 determines decreased β-cell mass [2,3].

Interestingly, in human pancreatic islet cells, the presence of a common IRS-1 polymorphism (Arg972) leads to defective insulin granule maturation, lower insulin secretion and increased apoptosis [4]. Recent studies have shown that, in the case of type 2 diabetes in humans, the islets show reduced expression of several of the molecules involved in early steps of insulin signaling, including IRS-1 and -2 [5].

The use of insulin in the therapy of people with type 2 diabetes is often necessary to rapidly improve glycaemic control at diagnosis or to achieve acceptable glucose levels later in the natural history of the disease [6]. Among the several insulin formulations available, insulin glulisine, a rapid-acting insulin analogue, appears to have peculiar effects on insulin signaling. The molecule has been shown to protect insulin-1 (INS-1) cells from immunological (cytokines) and metabolic (palmitic acid) insults [7], effects that appear to be mediated, at least in part, by preferential activation of IRS-2, a molecule with known anti-apoptotic action [7]. However, no information is currently available on whether glulisine confers any specific advantages on β-cell function and turnover in humans. Therefore, the present study examined the direct effects of glulisine on insulin signaling in human pancreatic islets, and compared them with those of regular human insulin (RHI) and another rapid-acting insulin analogue, insulin aspart.

The primary objective of this study in vitro was to assess the effects of glulisine compared with those of aspart and RHI on the function and molecular properties of isolated human pancreatic islets.

2. Materials and methods

2.1. Islet isolation and culture

Human pancreatic islets were prepared by collagenase digestion and density-gradient purification from the pancreata of multiple organ donors with the approval of our local ethics committee, as previously described [4,8,9]. To evaluate whether or not different rapid-acting insulin analogues affect insulin-signaling pathways differently, isolated islets were incubated for 48 h in M199 culture medium with and without glulisine, aspart and RHI at doses of 10 and 100 nmol/L. Glucose concentration in the incubation medium was maintained at either 5.5 (normal) or 22.2 (high) mmol/L of glucose. At the end of the incubation period, the following investigations were performed:

- insulin secretion during incubation by assessing levels of C-peptide using radioimmunoassay;
- acute glucose stimulation (3.3 and 16.7 mmol/L of glucose) by the batch incubation method;
- insulin, insulin receptor (IR), IRS-1, IRS-2 and phosphatidylinositol-3 kinase p110-alpha (PI3K) mRNA expression by quantitative real-time reverse-transcription polymerase chain reaction (quantitative real-time RT-PCR);
- phosphorylated IRS-2 protein expression by immunoblot analysis.

2.2. Insulin secretion studies

After a 45-min preincubation period at 3.3 mmol/L of glucose, groups of 30 islets of comparable size were kept at 37 °C for 45 min in Krebs–Ringer bicarbonate solution (KRB; 0.5% albumin, pH 7.4), containing 3.3 mmol/L of glucose. At the end of this period, the medium was completely removed and replaced with KRB containing either 3.3 or 16.7 mmol/L of glucose for 45 min. The medium was then again removed, and samples (500 µL) from the different incubation conditions were stored at −20°C until insulin concentrations were measured by immunoradiometric assay (IRMA; Pantec Forniture Biomediche, Turin, Italy).

2.3. Molecular studies

Quantitative gene expression of the molecules involved in insulin signaling (IR, IRS-1, IRS-2, PI3K) was assessed by quantitative real-time RT-PCR. Total RNA was extracted from...
the islets using RNeasy Protect Mini Kits (QIAGEN) and quantified by absorbance at A260/A280 (ratio > 1.65) nm in a PerkinElmer spectrophotometer. mRNA integrity was determined by electrophoresis in 1.0% agarose gels by ethidium bromide staining. The genes of interest were obtained from GenBank (National Center of Biotechnology Information), and the primer and probe sequences were purchased from PE Applied Biosystems (Pre-Developed TaqMan Assay Reagents Control Kits). PCR amplifications were performed in a total volume of 25 µL containing 2 µL of cDNA sample, 200 nmol/L of each primer, 100 nmol/L of the corresponding probes and 12.5 µL of TaqMan Universal PCR Master Mix. For each reaction, the polymerase was activated by preincubation at 95 °C for 10 min. Amplification was then performed over 40 cycles at 95 °C for 15 s and at 60 °C for 60 s. The quantity of each cDNA sample was normalized to the housekeeping gene for β-actin.

Immunoblot studies were performed as previously described [10,11]. Aliquots of islet cell lysates, obtained from 400 hand-picked islets and containing 200 µg of protein, were immunoprecipitated by incubation with antibodies against total and phosphorylated IRS-2 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) proteins. After immunoprecipitation, the bound antibodies were detected, using procedures carried out according to the manufacturer’s instructions (ECL, Amersham Biosciences, Buckinghamshire, UK). Bands of interest were quantified by a densitometer (GS-690, Bio-Rad Laboratories, Hercules, CA, USA), using MultiAnalyst/PC-PC Software for Bio-Rad’s Image Analysis Systems, Version 1.02 (Bio-Rad Laboratories).

2.4. Data analysis

Results were expressed as means ± standard deviation. Data were compared using two-tailed Student’s t test or analysis of variance (ANOVA), followed by the Bonferroni correction, as appropriate.

3. Results

3.1. Donor characteristics

Four donors without diabetes (one man and three women; age: 70 ± 13 years; body mass index: 24.7 ± 1.9 kg/m²) provided the pancreatic tissue that was used for the preparation of isolated islets.

3.2. Insulin secretion

At the end of the 48-h incubation, in the absence of exogenous added insulin, C-peptide levels were 1287 ± 540 pmol/L in the medium containing 5.5 mmol/L of glucose, and 3130 ± 1666 pmol/L in the medium containing 22.2 mmol/L of glucose (P < 0.05). The presence of glulisine, aspart or RHI had no significant effect on C-peptide levels (data not shown).

When acute glucose stimulation studies were performed, islets cultured in the presence of normal glucose showed significantly higher insulin secretion in response to an acute challenge with 16.7 mmol/L of glucose compared with 3.3 mmol/L of glucose (P < 0.05; Fig. S1; see supplementary material associated with this article online). Compared with islets preexposed to normal glucose, insulin secretion from islets preexposed to high glucose levels was significantly increased in response to a challenge with 3.3 mmol/L of glucose (P < 0.05), and was significantly decreased in response to 16.7 mmol/L of glucose (P < 0.05) (Fig. S1). Correspondingly, the stimulation index was significantly higher in islets preexposed to low glucose (Fig. S2; see supplementary material associated with this article online). The addition of glulisine, aspart or RHI at doses of either 10 or 100 nmol/L did not affect the stimulation index (Fig. S2; see supplementary material associated with this article online).

3.3. Molecular studies

In islets preexposed to normal glucose (5.5 mmol/L), the expression of IRS-1 and IRS-2 was not significantly affected by treatment with any insulin at any dose (Fig. 1). In contrast,
the expression of IR was reduced by low RHI, while that of PI3K was enhanced by both low and high concentrations of glulisine and aspart, and by high levels of RHI (Fig. 1). The effects of treatment with glulisine, aspart and RHI on islets cultured at 22.2 mmol/L of glucose are shown in Fig. 2. The expression of IRS-1 and IRS-2 was not significantly affected by treatment with any insulin at any dose, while the transcription of IR was increased by both low and high concentrations of aspart and RHI, but not by glulisine at either dose. High concentrations of all three insulin analogues increased the expression of PI3K, although this was only significant for glulisine (Fig. 2).

Compared with islets not exposed to any insulin, islets treated with all three tested insulins showed significant increases in IRS-2 phosphorylation ($P < 0.05$ by ANOVA; Fig. 3). Both glulisine and RHI demonstrated significantly greater effects compared with aspart ($P < 0.05$, with Bonferroni correction).

4. Discussion

This study confirmed the influence of differential exposures to glucose on insulin secretion and the stimulation index. Compared with islets preexposed to normal glucose levels, insulin secretion from islets preexposed to high glucose levels was significantly decreased following an acute challenge with glucose at levels of 16.7 mmol/L. Correspondingly, the stimulation index was significantly lower than for islets precultured in normal glucose, illustrating the effects of glucotoxicity in these cells. The introduction of exogenous insulin did not affect the secretion of insulin in response to glucose, suggesting that, at least under our present experimental conditions, preexposure to glulisine, aspart or RHI does not affect the complex mechanisms leading to the release of insulin granules.

In contrast, glulisine, aspart and RHI were found to have differential effects on the expression of proteins involved in the early steps of the insulin-signaling pathway in isolated human pancreatic islets. These include significantly higher levels of phosphorylated IRS-2 expression following exposure to glulisine compared with exposure to aspart, and a varying significance of the increases in expression of IR and/or PI3K in islets cultured with each of the exogenous insulins.

The overall action of each insulin on islets was found to be dependent on the degree of glucose exposure: in islets cultured in high glucose levels, only glulisine at 100 nmol/L significantly increased gene expression of PI3K, while aspart and RHI led to significant increases in IR gene expression, which was not seen in islets cultured in normal glucose levels. The potential clinical relevance of this observation in diabetes, a condition with wide blood glucose fluctuations and a spectrum of plasma insulin concentrations, remains to be explored.

Relatively few studies have evaluated the effects of insulin analogues on insulin signaling in either $\beta$ cells or other tissues,
with most studies conducted in cell lines. Of note, in INS-1 cells, glulisine was reported to induce prominent IRS-2 activation without activating IRS-1 and, thus, exerted anti-apoptotic activity against cytokines and palmitic acid. In contrast, aspart, lispro and RHI preferentially activated IRS-1. This was associated with marked inhibition of caspase-3 activity and nucleosomal release compared with RHI, aspart and lispro [7]. The authors attributed the selectivity of glulisine in activating IRS-2 to the relative expression of the insulin-like growth factor (IGF)-1 receptor in these insulinoma cells, and suggested that glulisine might activate the IGF-1 receptor to prevent induced apoptosis in these cells via the IRS-2 pathway. Nevertheless, the IRS-2 signaling pathway plays a pivotal role in the survival of β cells, as IRS-2-deficient mice are overtly diabetic, with reduced β-cell mass and impaired β-cell function [12,13], whereas IRS-1-deficient mice show growth retardation and mild insulin resistance, with compensatory β-cell expansion [14].

Interestingly, the authors also found that AspB10 and RHI were much less effective at inhibiting apoptosis despite IRS-2 activation comparable to that with glulisine, indicating that the effects of glulisine may be mediated through another pathway and may be partly independent of IGF-1-like activity. Indeed, it is clear that further studies are required to determine the precise mechanism by which glulisine protects against apoptosis in INS-1 and β cells.

Furthermore, in L6 skeletal muscle cells, the effects of lispro on time-dependent phosphorylation of IRS-1, p70 ribosomal S6 kinase, and ERK1/2 were nearly identical to those of RHI [15]; the addition of wortmannin, a PI3K inhibitor, suppressed the effects of lispro and RHI. The signaling characteristics of insulin glargine, insulin detemir and RHI have also been assessed in 3T3-L1 adipocytes, L6 myotubes, hepatocytes and vascular smooth muscle cells. Insulin glargine and RHI had similar effects on signaling pathways, with comparable dose-dependent phosphorylation of IR, IRS-1, Akt, GSK3 and p44/42 MAPK in all four cell lines. In contrast, the effects of detemir were lower in all cell lines assessed, which appeared to be due to the presence of albumin in the culture conditions, given the dose–response relationship between bovine serum albumin concentration [16] and the maximum responsiveness of detemir at the lowest albumin concentration. A study in vivo in a murine model showed unaltered insulin-signaling activation following acute glulisine treatment, compared with RHI, in liver and muscle tissues [17]. More recently, glulisine and RHI were tested with mouse mammary cells [18]. Although similar activation of ERK1/2 was observed, activation of Akt/PKB by glulisine was weaker than with RHI. As Akt/PKB is an effector of the PI3K pathway, thereby mediating the metabolic effects of insulin, the authors studied induction of hexokinase-2 in MCF7 cells, and hexokinase-2 and hexokinase-4 in HepG2 cells, by the two insulins. They found that the studied genes were not significantly induced by RHI and glulisine in those cell lines.

In a recently published report [19], Sciaccà et al. studied the effects of short- and long-acting insulin analogues on engineered cell models transfected with IR isoform A, IR isoform B and IGF-1 receptor. Minor differences were observed regarding the action of short-acting insulin analogues on receptor phosphorylation. However, in the presence of IR isoform B, lispro and glulisine (but not aspart) phosphorylated Akt less than insulin, and ERK phosphorylation was significantly reduced with glulisine.

Overall, these data consistently suggest the possibility that the various insulin analogues may differ in their modulation of β-cell function, despite some differences observed in certain experimental models—possibly due to the variety of cells and tissues studied and the length of exposure to insulin. Thus, glulisine may exert a β-cell protective effect via the up-regulation of IRS-2 in β cells and by suppressing β-cell apoptosis. In this case, the relevance of these effects in vivo need to be further assessed by specifically designed studies.

Conflicts of interest statement

This study was sponsored by Sanofi-Aventis. The authors have no additional conflicts of interest to disclose.

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Appendix A. Supplementary material

Supplementary material (Figs. S1 and S2) associated with this article can be found at http://www.sciencedirect.com, at doi:10.1016/j.diabet.2010.12.002.

References


