Glucose-Dependent Promotion of Insulin Release From Mouse Pancreatic Islets by the Insulin-Mimetic Compound L-783,281

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An insulin-mimetic compound (L-783,281) was used in an attempt to determine the role of the β-cell insulin receptor (IR) on insulin release. Islets were isolated from C57Bl/6j mice and cultured for 1 to 4 days. Insulin release from individual islets perfused in the presence of 3 mmol/l glucose was 10.5 ± 1.4 pg/min. Addition of 10 μmol/l L-783,281 had no effect on the rate of insulin secretion. When L-783,281 was added to perfusion medium containing 11 mmol/l glucose, the insulin-mimetic compound significantly increased insulin release. Insulin release from the isolated islet is pulsatile. In the presence of 3 mmol/l glucose, addition of L-783,281 significantly decreased the frequency of the oscillations from 0.35 ± 0.03 to 0.22 ± 0.04 oscillations/min. Addition of L-783,281 to perfusion medium containing 11 mmol/l glucose had no effect on the frequency of the insulin pulses (0.30 ± 0.05 oscillations/min). The results indicate that activation of the β-cell IR by L-783,281 augments insulin release in the presence of a stimulatory glucose concentration. At nonstimulatory glucose concentrations, activation of the β-cell IR may affect mechanisms related to the frequency of the insulin pulses. Diabetes 51 (Suppl. 1):S50–S52, 2002

Glucose is the prime secretagogue of insulin release from the pancreatic β-cell, and the secretion of insulin is dose-dependent on the metabolism of glucose (1). However, other factors also influence glucose-induced insulin release. It has long been assumed that insulin, like other hormones, would have an autocrine effect feeding back on its own secretion. The binding capacity of insulin to the β-cell has been described (2,3) and was determined to be analogous to the insulin receptor (IR) found in the target tissue of the hormone (4–6). With subsequent documentation of other components of the IR signaling pathway, such as insulin receptor substrates (IRS-1 and IRS-2) (6–8) and phosphatidylinositol 3-kinase (PI3-K) (9,10) in the β-cell, an autocrine role of the hormone via IR signaling was suggested. It was of fundamental importance to investigate how β-cell IR activation affects insulin release. By amperometric analysis, application of insulin to β-cells was claimed to enhance exocytosis as detected by 5-hydroxytryptamine (11). It has proved difficult to perform direct measurement of how insulin release is affected by IR activation in islets of Langerhans. However, access to an insulin-mimetic compound (L-783,281) and a sensitive assay for the hormone have made it possible to study the effects of IR activation on basal and glucose-stimulated insulin release in mouse pancreatic islets. Whereas L-783,281 enhanced glucose-induced insulin release without altering the frequency of the pulses, the frequency of the insulin pulses was modulated by the compound at basal glucose.

RESEARCH DESIGN AND METHODS

Materials. Reagents of analytical grade and deionized water were used. Collagenase, HEPES, and bovine serum albumin (fraction V) were obtained from Boehringer Mannheim GmbH (Mannheim, Germany). Tetramethylbenzidine and insulin-peroxidase came from Sigma (St. Louis, MO). The rat insulin standard was from Novo Nordisk (Bagsvaerd, Denmark). Immunoglobulin G–certified microtiter plates were purchased from Nunc (Roskilde, Denmark). The mouse insulin antibodies were raised in guinea pigs in our laboratory. The insulin analog L-783,281 was obtained from Merck.

Preparation and culture of islets. Pancreatic islets were collagenase isolated from C57Bl/6j mice (B&K, Sollentuna, Sweden). The islets were cultured for 1 to 4 days in 11 mmol/l glucose in RPMI culture medium supplemented with 10% fetal calf serum, 100 IU/ml penicillin, 100 μg/ml streptomycin, and 30 μg/ml gentamicin. The experiments were approved by the local animal ethics committee.

Perfusion of islets. The kinetics of insulin release were studied essentially as described previously (12). A single islet was placed in a 10-μl chamber (thermostat at 37°C) and perifused at a constant flow rate with medium containing 3 mmol/l glucose. The experiments were performed in medium supplemented with 1 mg/ml albumin and containing (in mmol/l): NaCl 125, KCl 5.9, MgCl2 1.2, CaCl2 1.3, and HEPES 25, titrated to pH 7.4 with NaOH. The flow rate was kept constant throughout each experiment with the aid of a peristaltic pump placed before the islet. Flow variation of 150 to 200 μl/min was allowed between experiments. After 60 to 75 min of introductory perfusion, the perfusate was collected in 20-s fractions directly into microtiter plates.

Insulin measurements. Insulin was assayed by a competitive enzyme-linked immunosorbent assay (ELISA) with the insulin antibody immobilized directly onto the solid phase. Amounts of insulin down to 100 amol were obtained from linear standard curves in semilogarithmic plots. The insulin-mimetic compound significantly increased insulin release. Insulin-mimetic compound (L-783,281) was used in an attempt to determine the role of the β-cell insulin receptor (IR) on insulin release. Islets were isolated from C57Bl/6j mice and cultured for 1 to 4 days. Insulin release from individual islets perfused in the presence of 3 mmol/l glucose was 10.5 ± 1.4 pg/min. Addition of 10 μmol/l L-783,281 had no effect on the rate of insulin secretion. When L-783,281 was added to perfusion medium containing 11 mmol/l glucose, the insulin-mimetic compound significantly increased insulin release. Insulin release from the isolated islet is pulsatile. In the presence of 3 mmol/l glucose, addition of L-783,281 significantly decreased the frequency of the oscillations from 0.35 ± 0.03 to 0.22 ± 0.04 oscillations/min. Addition of L-783,281 to perfusion medium containing 11 mmol/l glucose had no effect on the frequency of the insulin pulses (0.30 ± 0.05 oscillations/min). The results indicate that activation of the β-cell IR by L-783,281 augments insulin release in the presence of a stimulatory glucose concentration. At nonstimulatory glucose concentrations, activation of the β-cell IR may affect mechanisms related to the frequency of the insulin pulses. Diabetes 51 (Suppl. 1):S50–S52, 2002

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IR, insulin receptor.

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RESULTS

Insulin release from individual islets perifused in the presence of 3 mmol/l glucose was 10.5 ± 1.4 pg/min and pulsatile (0.35 ± 0.03 oscillations/min) (Fig. 1). When 10 μmol/l L-783,281 was added to the perfusion medium, there was no change in the rate of secretion, but the pulse frequency decreased significantly (P < 0.02) to 0.22 ± 0.04 oscillations/min. Islets were also perifused in the presence of 11 mmol/l glucose (Fig. 2). Upon introduction of the higher glucose concentration, an initial accentuated peak of insulin was followed by insulin pulses with declining amplitudes (not shown). After 30 min, L-783,281 was added to the perfusion medium containing 11 mmol/l glucose. The addition of the compound increased insulin release from 15.6 ± 4.8 to 64.3 ± 16.9 pg/min. The effect of L-783,281 on the pulse frequency was heterogeneous, and no statistical change could be established.

DISCUSSION

In the present study, we show that the insulin-mimetic compound L-783,281 increases the rate of glucose-stimulated but not basal insulin release from the individual islet of Langerhans measured by an immunological method. At basal but not stimulatory glucose concentration, the compound clearly modulated the frequency of the pulsatile insulin release. L-783,281 was used to obtain β-cell IR activation without applying insulin, which would not have been distinguished from secreted insulin by the assay. The insulin mimic, which was recently described (14), is a nonpeptidyl metabolite derived from a fungal extract that activates the IR tyrosine kinase, stimulates insulin gene expression, and decreases blood glucose levels in murine models of type 2 diabetes (14,15). In a previous study, insulin-stimulated exocytosis in β-cells measured by amperometry was similarly stimulated at basal and stimulatory glucose concentrations (11). Whether the conflicting results with regard to the glucose-dependency of insulin stimulation on insulin release in the present study depend on the usage of L-783,281 rather than insulin remains to be elucidated.

The mechanisms involved in insulin-induced insulin release have been proposed to involve regulation of the intracellular Ca²⁺ homeostasis via SERCA3 (SERCA, sarco/endoplasmic reticulum calcium ATPase) (15,16). This concept was supported by findings of insulin-induced cytoplasmic Ca²⁺ mobilization from the endoplasmic reticulum (17). The cytoplasmic Ca²⁺ concentration ([Ca²⁺]c) plays a pivotal role in the regulation of glucose-induced insulin release (18). Oscillations in [Ca²⁺]c are closely followed by pulses of insulin release (19). The [Ca²⁺]c oscillations, which appear at stimulatory glucose concentrations, have been found to depend on paracrine factors promoting mobilization of Ca²⁺ from intracellular stores (20). The L-783,281–induced change in pulse frequency was observed at basal glucose, when [Ca²⁺]c is nonoscillatory. If β-cell IR activation at basal glucose also induces changes in [Ca²⁺]c, these changes may be important for the observed alteration in frequency. Insulin resistance in type 2 diabetes may be associated with impaired handling of Ca²⁺ in the endoplasmic reticulum (15,16). Such impaired handling may contribute to the deranged plasma insulin pattern observed in such individuals (21). In healthy subjects, blood insulin levels fluctuate regularly (22).
The importance of the expression of the β-cell IR was also apparent when the receptor was selectively knocked out in the β-cell in the so-called βIRKO mouse, which displays a secretory response to glucose similar to that of type 2 diabetes (23). In contrast to other autocrine feedback systems, the β-cells seem to signal via positive feedback. This seemingly dangerous self-promoting secretion of the hypoglycemic hormone may be explained by the present observation of glucose dependency of the β-cell IR-mediated effect.

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