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Pioglitazone acutely influences glucose-sensitive insulin secretion in normal and diabetic human islets

Fan Zhang, Åke Sjöholm, Qimin Zhang *

Karolinska Institutet, Department of Internal Medicine, Stockholm South Hospital, SE-11883 Stockholm, Sweden

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Abstract

We have studied acute effects of the PPARγ agonist pioglitazone *in vitro* on human islets from both non-diabetic and type 2 diabetic subjects. In 5 mM glucose, pioglitazone caused a transient increase in insulin secretion in non-diabetic, but not diabetic, islets. Continuous presence of the drug suppressed insulin release in both non-diabetic and diabetic islets. In islets from non-diabetic subjects, both high glucose and tolbutamide-stimulated insulin secretion was inhibited by pioglitazone. When islets were continuously perifused with 5 mM glucose, short-term pretreatment with pioglitazone caused approximately 2-fold increase in insulin secretion after drug withdrawal. Pioglitazone pretreatment of diabetic islets restored their glucose sensitivity. Examination of cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) in non-diabetic islets revealed slight Ca^{2+} transient by pioglitazone at 3 mM glucose with no significant changes at high glucose. Our data suggest that short-term pretreatment with pioglitazone primes both healthy and diabetic human islets for enhanced glucose-sensitive insulin secretion.

Keywords: Pioglitazone; Peroxisome proliferator-activated receptor (PPAR); Glucose sensitivity; Insulin secretion; Cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$); Human islets; Diabetes

Peroxisome proliferator-activated receptors (PPARs) are nuclear receptors, composed of three major isoforms (α, β/δ, and γ) that have been identified to date [1,2]. The receptor isoforms show tissue and cell specificity, and exert related but distinct functions upon activation [3–7]. Among the PPAR isoforms, PPARγ is the best characterized and found to play an important role in the regulation of energy homeostasis [1,2,8–10]. Thiazolidinediones (TZDs), agonists of PPARγ, reduce insulin resistance and thus influence free fatty acid flux and blood glucose levels. The glucose-lowering effect of TZDs is attributed to increased peripheral glucose disposal and decreased hepatic glucose output [8,11,12]. Synthetic ligands for PPARγ are of particular interest for treating patients with type 2 diabetes because they restore the sensitivity of the tissues to insulin [8,9,11]. Pioglitazone is an orally administered insulin-sensitizing TZD agent that activates PPARγ, leading to the increased transcription of genes encoding various proteins regulating glucose and lipid metabolism [3,11]. In contrast to its action on insulin target tissues, little is known about the direct action of the PPARγ agonist on pancreatic β-cell function and the β-cell sparing effects noted in clinical trials have generally been assumed to be secondary to the decreased insulin resistance. In the present study, we have studied the direct actions of the PPARγ agonist pioglitazone on insulin secretion and Ca^{2+} handling in human islets from non-diabetic and diabetic subjects during short-term treatment *in vitro*.

Materials and methods

Pioglitazone was graciously donated by Takeda Pharmaceuticals North America, Inc. (Lincolnshire, IL). Fura-2/acetoxymethylester (Fura-2/AM) was from Sigma (St. Louis, MO). Bio-Gel P-4 (fine, 65 ± 20 μm, wet) was from Bio-Rad Laboratories (Hercules, CA). Insulin ELISA kits were from Mercodia (Uppsala, Sweden).

* Corresponding author. Fax: +46 8 6163287.
E-mail address: Qimin.Zhang@sos.ki.se (Q. Zhang).

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Human islets

Pancreatic islets from adult non-diabetic or diabetic donors were obtained from the Uppsala University Hospital facility for isolation of human islets from Scandinavian brain-dead donors. The 56-year-old diabetic donor was diagnosed with type 2 diabetes for 10 years and received insulin 90 U daily before death with BMI 33, HbA_{1c} 9.5%, and C-peptide level of 0.3 nmol/l. The normal islets were from adult brain-dead non-diabetic patients. The islets were maintained in RPMI-1640 tissue culture medium in the presence of 5.5 mM glucose, supplemented with 10% (v/v) FBS, 2 mM L-glutamine, 100 IU/ml penicillin, and 100 μg/ml streptomycin, and were used for experiments 8 days after isolation. All experiments were approved by the Karolinska Ethics Committee.

Insulin secretion

Column perifusion. Dynamics of insulin secretion was studied by column perifusion as described [13,14]. About 100 human islets were carefully mixed with a small volume of pre-wetted Bio-Gel P-4, placed on top of each of the columns, and perifused with KRBH (Krebs–Ringer bicarbonate Hepes) buffer containing (in mM): 135 NaCl, 3.6 KCl, 5 $NaHCO_3$, 0.5 NaH_2PO_4, 0.5 $MgCl_2$, 1.5 $CaCl_2$, and 10 Hepes, pH 7.4, with 0.1% BSA.

Batch incubation. Islets were washed three times in KRBH buffer. Equal number of islets of comparable size was placed in each well of a 24-well plate, pre-added with 1 ml of the buffer in the presence of pioglitazone or DMSO at 3 mM glucose. After pre-incubation for 10 min at 37 °C, glucose or glucose with tolbutamide was added in the corresponding wells and incubated for 20 min. The medium was collected and centrifuged, the supernatant was for insulin assay. The islets were collected and lysed for protein assay (Bio-Rad).

Measurement of $[Ca^{2+}]_i$. Fura-2 loaded cells were perifused with KRBH buffer in the presence of 3 mM glucose and 0.1% BSA, at 37 °C. Measurements of $[Ca^{2+}]_i$ were performed on single cells prepared from islets as described [15,16] using a time-sharing spectrofluorometer (RM-5 System, PhotoMed, Denmark).

Results

Bimodal effects of pioglitazone on glucose-sensitive insulin secretion in human islets

When human islets from non-diabetic subjects were perifused with 5 mM glucose, administration of 10 μM pioglitazone elicited an approximately 20% transient increase in insulin secretion, followed by a suppression of insulin release from the islets (Fig. 1). The inhibitory effect of pioglitazone remained until withdrawal of the drug, resulting in up to 40% of decrease in insulin secretion, compared to those before the drug addition. Immediately after withdrawal of pioglitazone, insulin secretion was increased approximately threefold compared to the levels during pioglitazone administration, and twofold compared to the levels before addition of the drug. The same response pattern to pioglitazone was noted in all experiments performed using islets from different donors, but not in those with buffer changes alone.

Pioglitazone inhibits glucose-induced insulin secretion in normal human islets

The effect of pioglitazone on insulin secretion was further investigated by acute exposure of human islets to the drug in the presence of low and high glucose (Fig. 2). Compared to the inhibitory effect of pioglitazone on insulin secretion at 5 mM glucose, the drug did not result in

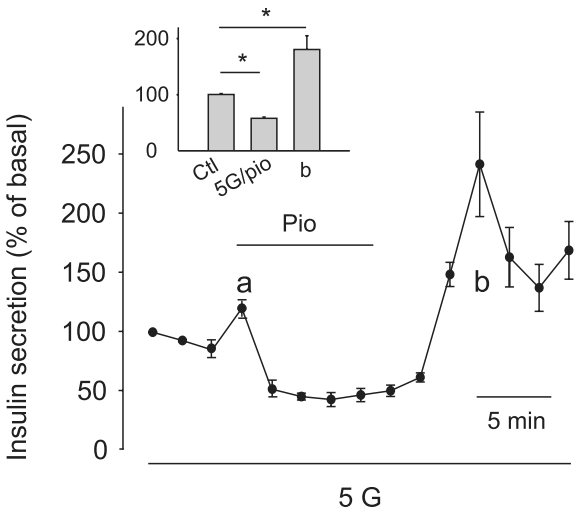

Fig. 1. Bimodal effects of pioglitazone on insulin secretion dynamics in normal human islets. Human islets from non-diabetic donors were packed in a micro-column and perifused with KRBH buffer containing 0.1% BSA and 5 mM glucose. Pioglitazone (Pio; 10 μM) or vehicle (DMSO)-containing buffer (as control) was introduced. Data show dynamic changes in insulin secretion from four separate experiments using islets from four different subjects. The average of the insulin content in the fractions collected before the drug addition (Control) was considered as 100%. Insulin content in each fraction was divided by the average. Results are expressed as percentage changes of insulin secretion. The insert figure shows the suppressed insulin secretion during perifusion with pioglitazone, and enhanced insulin secretion after withdrawal of the drug (peak b) in the four experiments. $^*P < 0.05$, by ANOVA.

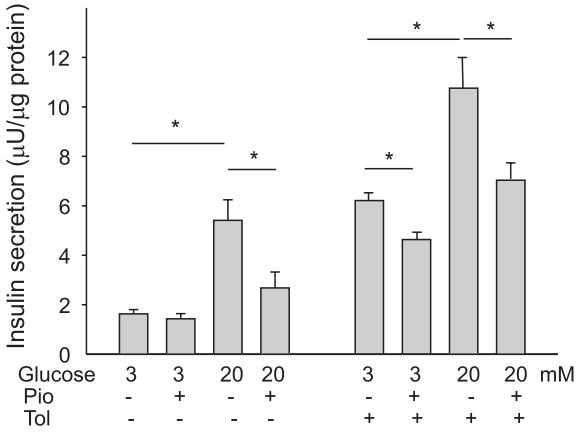

Fig. 2. Effects of pioglitazone on glucose- and tolbutamide-stimulated insulin secretion in human islets. Human islets from non-diabetic donors were pre-incubated in the presence of pioglitazone (Pio) or vehicle (DMSO) for 10 min in KRBH buffer containing 0.1% BSA and 3 mM glucose at 37 °C, followed by addition of glucose (20 mM) or glucose with tolbutamide (Tol; 100 μM) in the corresponding wells. Insulin secretion from the islets in each well was normalized by protein content measured in the lysed islets. Results from four separate experiments using islets from three different subjects are shown and expressed as means ± SEM. $^*P < 0.05$ by ANOVA.