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Short communication

Expression of peroxisome proliferator-activated receptor γ (PPARγ) in normal human pancreatic islet cells

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Abstract

Aims/hypothesis. Thiazolidinediones are reported to improve pancreatic islet morphology and beta-cell function in rodents, supporting the hypothesis of a direct action of thiazolidinediones on endocrine islet cells. In this study we examined the expression of the peroxisome proliferator-activated receptor γ, a nuclear receptor that is activated by naturally occurring fatty acids and synthetic thiazolidinediones, in normal human endocrine pancreatic cells.
Methods. Human islets were isolated from pancreata harvested in ten brain-dead lean non-diabetic adult donors. We analysed the gene and protein expression of the human peroxisome proliferator-activated receptor γ and evaluated the effects of peroxisome proliferator-activated receptor γ agonist on insulin secretion in human islet preparations.
Results. The RT-PCR carried out on total RNA from four distinct human islet preparations demonstrated the presence of peroxisome proliferator-activated receptor γ mRNA. Western blot analysis showed the consistent expression of peroxisome proliferator-activated receptor γ protein. Peroxisome proliferator-activated receptor γ was shown to be present in all three endocrine cell types studied (alpha, beta and delta cells) by immunohistochemistry.
Conclusion/interpretation. We found that peroxisome proliferator-activated receptor γ is highly expressed in human islet endocrine cells, both at the mRNA and protein levels. These results support the hypothesis of a direct influence of peroxisome proliferator-activated receptor γ agonist on human pancreatic endocrine cells. [Diabetologia (2000) 43: 1165–1169]

Keywords Type II diabetes, peroxisome proliferator-activated receptor γ, human pancreas, islets, beta cell, thiazolidinediones, troglitazone, rosiglitazone.

Peroxisome proliferator-activated receptor γ (PPARγ) is a nuclear receptor having a key role in adipogenesis [1]. The PPARγ has been identified as the receptor for the thiazolidinediones, a new class of oral antidiabetic agents which improves glycaemic control by lowering peripheral insulin resistance. Apart from its effects on peripheral insulin resistance, the first thiazolidinedione to be approved for clinical use, troglitazone, has been shown to restore the beta-cell response to an oscillatory glucose infusion, to enhance insulin secretion rates when adjusted to insulin sensitivity index [2] and to decrease the proinsulin:insulin ratio [3]. Several studies both in vivo [4] and in vitro [5] confirmed that treatment with thiazolidinediones improves pancreatic islet morphology and beta-cell function, further supporting the hypothesis of a direct action of thiazolidinediones on endocrine islet cells. Because PPARγ is the only known receptor for thiazolidinediones, its expression in human endocrine pancreatic cells needs to be shown to support the clinical relevance of this hypothesis. The expression of PPARγ has been ob-

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Abbreviations: AEC, 3-amino-9-ethyl-carbazole; BCIP/NBT, 5-bromo-4-chloro-3-indoxyl phosphatase/nitro blue tetrazolium chloride; PPARγ, peroxisome proliferator-activated receptor γ; RAU, relative absorbance units; WAT, white adipose tissue; DMSO, dimethyl sulphoxide.

served in various tissues but has not been described in the human pancreas [6]. Therefore the goal of this study is to examine the expression of PPARγ at the mRNA and protein levels in normal human endocrine pancreatic cells.

Materials and methods

Materials. Rosiglitazone (BRL-49,653) was a gift from Dr R. Heyman at Ligand Pharmaceuticals (San Diego, Calif., USA).

Human tissues. Human pancreata ($n = 10$) were procured from brain-dead non-diabetic (mean $HbA_{1C} = 5.4 \pm 0.1$) and non-obese (mean BMI = 27 ± 1) adult donors (mean age 41.3 ± 3.5) in accordance with French regulations. Pancreata were stored at 4 °C in Euro-Collins solution (Fresenius, Bad Homburg, Germany) until islets were isolated with the semi-automatic method of Ricordi and purified with Euroficoll (Ficoll 400 DL, Sigma Aldrich, Saint Quentin Fallavier, France) or Histopaque (Sigma Aldrich) discontinuous density gradients following a multilayer test gradient. Purity of preparations was visually estimated, usually as greater than 80 % after staining islets with dithizone. Islet preparations were cultured in CMRL-1066 (Gibco BRL, Paisley, Scotland) supplemented with 2 % Ultroser G (Gibco BRL), 100 UI/ml penicillin, 100 μg/ml streptomycin, 50 μg/ml gentamycin and 1 μg/ml amphoterycin B. Mesenteric white adipose tissues (WAT) and colon biopsy specimens obtained during surgery from non-diabetic and non-obese patients were included as control for RNA and protein analysis. All tissues samples were immediately frozen in liquid nitrogen.

Antibodies. The PPARγ antibody used in this study was a polyclonal IgG developed against a peptide corresponding to amino acids 20–104 of human PPARγ. This antibody was highly specific for PPARγ and did not cross-react with PPARα or β as described previously [7]. Antibodies to islet specific hormones included polyclonal and monoclonal anti-insulin (Bio-Genex, Paris, France) and polyclonal anti-glucagon and anti-somatostatin (Dako, Carpinteria, Calif., USA).

Western blot analysis. Total protein from islet preparations ($n = 5$), mesenteric WAT ($n = 2$) and colon ($n = 2$) were obtained by homogenisation in extraction buffer composed of Triton (2 %) to which a protease inhibitor cocktail was added [7]. Separation of total proteins (50 μg per lane), transfer to nitro-cellulose membranes and immunodetection of PPARγ were done as described [7]. The complex was revealed by chemiluminescence according to the manufacturer's protocol (ECL; Amersham Life Science, Les Ulis, France).

mRNA analysis by RT-competitive PCR. Total RNA from islet preparations ($n = 4$), mesenteric WAT ($n = 9$) and colon ($n = 18$) were extracted using Tri-reagent (Euromedex, Souffelweyersheim, France) according to the manufacturer's instructions. First strand cDNA was generated from total RNA and PPARγ and β-actin cDNA were amplified by 40 competitive PCR cycles as described [8].

Immunohistochemistry. Pancreas samples ($n = 5$) were fixed for 18 h in 4 % paraformaldehyde, embedded in paraffin and sectioned (5 μm). After deparaffinisation and rehydration in ethanol, non-specific binding and endogenous peroxidases were blocked with 10 % goat serum and hydrogen peroxidase, respectively, when necessary. All antibodies were incubated 1 h at room temperature with the following dilutions 1/50 for anti-PPARγ, 1/100 for polyclonal anti-insulin, 1/750 for monoclonal anti-insulin, 1/400 for anti-glucagon and 1/500 for anti-somatostatin. For simple immunolabelling on adjacent sections, primary antibodies were revealed with the streptavidin-biotin-peroxidase system (HistoMark kit; Kirkegaard and Perry Laboratories, Gaithersburg, Md., USA) using the chromogen 3-amino-9-ethyl-carbazole (AEC; Sigma Aldrich). In double immunolabelling procedures, anti-PPARγ polyclonal antibody was visualised with the EnVision phosphatase mouse/rabbit kit (Dako) using the chromogen 5-bromo-4-chloro-3-indoxyl phosphatase / nitro blue tetrazolium chloride (BCIP/NBT; Dako) and anti-insulin monoclonal antibody with the Envision peroxidase mouse using AEC. Control sections were incubated with dilution buffer (PBS with 1 % BSA) instead of the primary antibody. In some sections nuclei were counterstained with Carazzi's haematoxylin.

In vitro insulin secretion. Rosiglitazone was dissolved in dimethyl sulphoxide (DMSO). After a 24-h culture, random samples of islet preparations containing 40 islets with an equivalent diameter of 150 μm were distributed in 96-well multiscreen assay system (Millipore, Strasbourg, France) [9] and cultured for 48 h in fresh medium supplemented with rosiglitazone (10^{-8}, 10^{-7}, 10^{-6} or 10^{-5} mol/l) or DMSO for controls. After a 48-h culture, a static incubation was done that consisted of a preincubation for 90 min (with 3.3 mmol/l glucose), followed by three incubations of 1 h with 3.3 mmol/l glucose (basal 1), 16.6 mmol/l glucose + 0.1 mmol/l 3-isobutyl-1-methylxanthine (IBMX) (stimulation) and 3.3 mmol/l glucose (basal 2), respectively, in KRB containing 0.5 % BSA. At the end of each incubation, KRB was collected. All samples were frozen at –20 °C until insulin secretion was evaluated (bi-insulin IRMA, Sanofi Diagnostics Pasteur, Marnes-la-Coquette, France). Basal insulin secretion was defined as the mean of basal 1 and basal 2 secretions. For each condition, the mean result of six experiments for each of three distinct islet preparations was considered.

Statistical analyses. Continuous variables are expressed as means ± SEM. Statistical analysis of insulin secretion values was by the ANOVA test (StatView, Abacus Concept, Berkeley, Calif., USA). Significance was set at $p < 0.05$.

Results

Expression of PPARγ protein and mRNA in the human pancreas. The expression of human PPARγ protein was analysed in human islet preparations and compared with other tissues expressing PPARγ protein. Western blot analysis of lysates of mesenteric WAT and colon revealed a band with an approximate molecular mass of 53,000 Mr, corresponding to the molecular weight of PPARγ (Fig. 1). Notably, lysates of islet preparations revealed a slightly higher band of approximately 55,000 Mr, most likely corresponding to a phosphorylated form of PPARγ. Expression of PPARγ in islets was approximately 2/3 of those detected in mesenteric WAT (Fig. 1). Expression of PPARγ remained unchanged in islets after 8 days of culture (data not shown). The RT-PCR confirmed

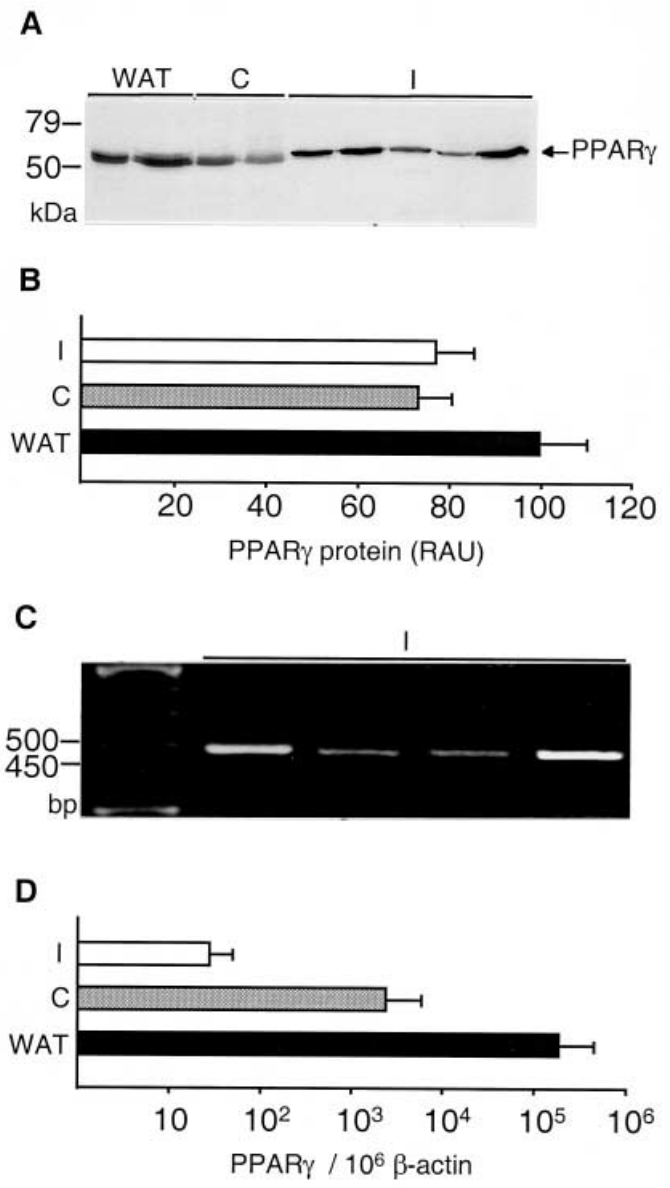

Fig. 1A–D. Expression of PPARγ in isolated islet preparations. **A** Total protein lysates from mesenteric white adipose tissue (WAT; $n = 2$), colon (C; $n = 2$), and islet preparation (I; $n = 5$) were analysed by western blot. The band of 53,000 Mr corresponds to the size of the PPARγ protein. **B** Quantitative and comparative representation of PPARγ protein concentrations in tissues shown in panel **A** obtained by densitometric analysis. Mean values are normalised to the value of mesenteric WAT and represented as relative absorbance units (RAU). **C** Total RNA from islet preparations was analysed by RT-PCR. PPARγ mRNA expression is visualised by the presence of a band corresponding to an amplification product of 475 bp. **D** Quantitative and comparitive representation of PPARγ mRNA expression in mesenteric WAT ($n = 9$), colon ($n = 18$) and islet preparations ($n = 4$) by RT-competitive PCR. Values are represented as the mean of the number of PPARγ cDNA molecules per 10^6 molecules of β-actin cDNA

the presence of PPARγ mRNA (product size 475 bp) in all islet preparations (Fig. 1). When quantified by RT-competitive PCR in four distinct pancreata the values of 4.06; 9.06; 9.93 and 85.62 PPARγ cDNA molecules per 10^6 β-actin cDNA molecules were obtained, with a mean of 27.17 ± 19.53. The mean concentration of PPARγ in the control tissues was $1.8 \times 10^5 \pm 2.6 \times 10^5$, cDNA molecules in the mesenteric WAT ($n = 9$) and $2.3 \times 10^3 \pm 3.3 \times 10^3$ cDNA molecules in colon ($n = 18$), per 10^6 molecules of β-actin cDNA (Fig. 1).

Localisation of PPARγ in human pancreatic tissue. Expression of PPARγ in pancreatic tissue was studied next by immunohistochemistry on human pancreatic sections. Simple immunolabelling of PPARγ using the same antibody as used for the western blots confirmed that this nuclear receptor was highly expressed in islets (Fig. 2). Immunolabelling on adjacent sections of glucagon, insulin and somatostatin, hormones that are specific to alpha, beta and delta cells, respectively, showed the presence of PPARγ in all three cell types of human islets (Fig. 2). Expression of PPARγ in beta cells was confirmed by double immunolabelling of PPARγ and insulin in the same pancreatic section (Fig. 2).

Functional studies. Influence of thiazolidinediones on insulin secretion was investigated using increasing concentrations of rosiglitazone. Basal and stimulated insulin release, defined in materials and methods, were 726.4 ± 86.6 μUI and 1410.0 ± 224.5 μUI in control medium, 731.3 ± 93.6 μUI and 1483.5 ± 203.6 μUI in the presence of 10^{-8} mol/l rosiglitazone; 719.7 ± 74.9 μUI and 1457.6 ± 188.6 μUI in the presence of 10^{-7} mol/l rosiglitazone; 733.5 ± 73.7 μUI and 1430 ± 170.7 μUI in the presence of 10^{-6} mol/l rosiglitazone; 705.2 ± 77.5 μUI and 1420.3 ± 200.8 μUI in the presence of 10^{-5} mol/l rosiglitazone. No statistical differences were observed in insulin concentrations between the control and rosiglitazone-treated cells ($p > 0.99$).

Discussion

In patients with Type II (non-insulin-dependent) diabetes mellitus, thiazolidinediones reduce peripheral insulin resistance, but also seem to improve the pattern of insulin secretion. Various rodent studies suggest that thiazolidinediones could exert a direct effect on beta cell morphology and function, by preventing pancreatic islet hyperplasia and beta cell hypertrophy, by restoring beta cell granulation and the intra-islet distribution pattern of the alpha and beta cell and by lowering islet fat content [4, 5]. In this study, we show that PPARγ is expressed in normal

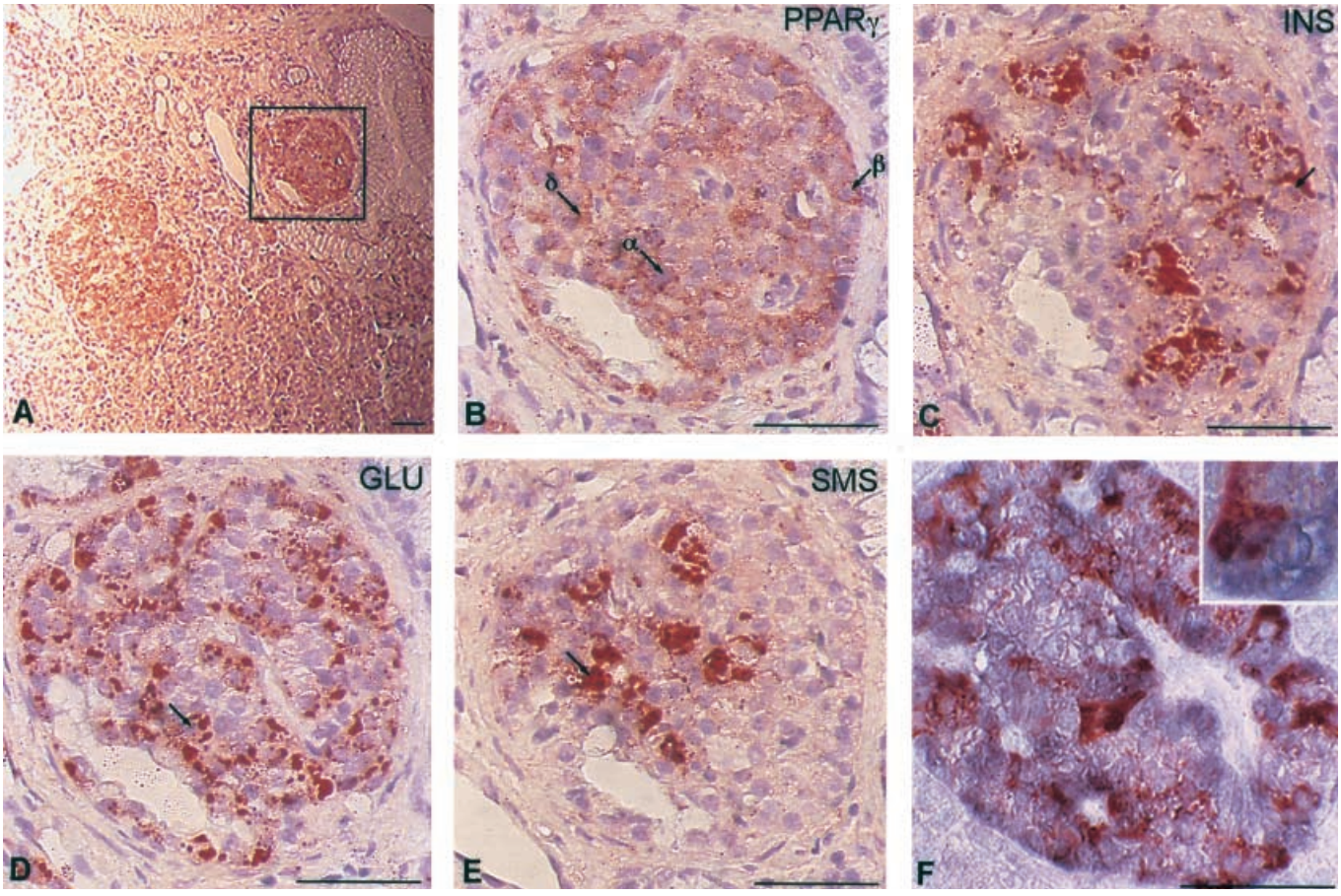

Fig. 2 A–F. Localisation of PPARγ in alpha, beta and delta cells of pancreatic human islets by immunohistochemistry. Simple immunolabelling of PPARγ (**A** and **B**), insulin (**C**), glucagon (**D**) and somatostatin (**E**) on adjacent sections with AEC (red), and double immunolabelling of PPARγ with BCIP/NBT (blue) and insulin with AEC (**F**). Arrows indicate cells in which PPARγ is co-localized with insulin (beta), glucagon (alpha) or somatostatin (delta). The bar represent 50 μm

human pancreatic islets at the mRNA and protein levels. Although documented in various tissues such as adipose tissue, large and small intestine, haematopoietic cells, kidney, liver and muscle [1], the expression of PPARγ has to date never been described in the human pancreas. Using RT-PCR we have shown the expression of PPARγ mRNA in human islet preparations, corroborating its previous detection in the rodent pancreas [10]. Although PPARγ mRNA was detected in all subjects studied, marked differences in expression were observed by RT-competitive PCR reflecting the highly variable characteristics of the organ donors. Western blot studies showed the consistent presence of high concentrations of PPARγ protein in all islet preparations. Important differences in protein concentrations and mRNA expressions of PPARγ were observed between WAT and islet preparations. This discrepancy is most likely attributed to a combination of several factors including the presence of enzymes influencing the stability of mRNA and protein and the difference in sensitivity of each of the techniques used. We confirmed by immunochemistry that PPARγ was preferentially localised in the endocrine islets within the pancreas. No difference was noted between the three endocrine cell types studied (alpha, beta and delta) which all co-expressed PPARγ.

When exposing human islet preparations obtained from these non-diabetic donors to various concentrations of rosiglitazone, we did not observe any effect on basal nor stimulated insulin secretion. These results confirm those obtained in the lean wild-type (+ / +) Zucker diabetic fatty rats [5]. Nevertheless, when studying islets of obese homozygous (*fa*/*fa*) Zucker diabetic fatty rats, these authors showed that thiazolidinediones restore the beta-cell insulin secretion pattern by lowering islet fat content [5], an effect potentially mediated by PPARγ.

We found the transcription and expression of PPARγ in human islet endocrine cells to be consistent. These findings further highlight the connection between PPARγ and glucose homeostasis [6] and support the hypothesis of a direct influence of thiazolidinediones on pancreatic beta cell homeostasis explaining their action on the insulin secretion pattern in diabetic patients.

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