Effect of PPAR-γ Activation and Inhibition on Glucose-Stimulated Insulin Release in INS-1e Cells

Eleonora Santini, Poupak Fallahi, Silvia Martina Ferrari, Antonio Masoni, Alessandro Antonelli, and Ele Ferrannini

Peroxisome proliferator–activated receptor (PPAR)-γ is expressed in human β-cells and in the rat β-cell line INS-1. Previous studies have suggested that PPAR-γ agonism (e.g., thiazolidinediones) enhances glucose-stimulated insulin secretion (GSIS) from islets or INS-1 cells. We tested the direct effect on insulin release by INS-1e of a PPAR-γ agonist (Ro4389679-000-001 at 0.2 and 0.4 μmol/l) and a PPAR-γ antagonist (SR202 at 0.2 and 0.4 mmol/l). Cells were incubated in 11 mmol/l glucose for 96 h and then challenged with 3.3, 7.5, 11.0, and 20.0 mmol/l glucose for 1 h. Under these control conditions, insulin concentrations in the medium rose from 19 ± 4 ng/ml (mean ± SE) to 82 ± 5, 107 ± 11, and 103 ± 10 ng/ml ($P < 0.0001$ by ANOVA). Preincubation for 48 h with the PPAR-γ agonist potentiated GSIS (to 154 ± 14 and 156 ± 12 ng/ml at 20 mmol/l glucose, $P < 0.01$). Cell insulin content was not altered by either acute glucose challenge or PPAR-γ agonist coincubation. Preincubation for 48 h with SR202 at the higher dose caused a 30% inhibition of GSIS, with no change in cell insulin contents. When cells were preincubated with 11 mmol/l glucose plus 1 mmol/l oleate, GSIS was significantly potentiated (by 30%, $P < 0.0001$); adding Ro4389679-000-001 or SR202 to these preincubations reduced GSIS to the respective levels seen in the absence of oleate ($P < 0.0001$ for both effects). In conclusion, INS-1e cells display a PPAR-γ tone that is symmetrically modulated and competitively stimulated by oleate. *Diabetes* 53 (Suppl. 3):S79–S83, 2004

Peroxisome proliferator–activated receptor (PPAR)-γ is a member of the nuclear receptor superfamily, which is expressed at a high level in adipose tissue and regulates its differentiation, thereby playing a key role in lipid metabolism. Studies of PPAR-γ function have identified direct affinity ligands, such as the antidiabetic agents thiazolidinediones (1,2). Thiazolidinediones increase the number of small adipocytes in obese Zucker rats (3); in humans, these compounds change fat distribution (4), reduce serum insulin levels, and enhance whole-body insulin sensitivity (4–7). Their antihyperglycemic effect is thought to principally relate to their action on insulin sensitivity. However, some studies indicate that thiazolidinediones may directly stimulate glucose-stimulated insulin secretion (GSIS) from pancreatic β-cells in vitro (8–10) and in vivo (11,12). Kawai et al. (13) have demonstrated that PPAR-γ is expressed in a rat insulinoma cell line, INS-1. Furthermore, human β-cells have recently been shown to express PPAR-γ2, thereby offering a direct therapeutic target for PPAR-γ agonists (14).

Free fatty acids (FFAs) are very important in the development of type 2 diabetes and may be associated with "lipotoxicity" (15–17); the importance of the chain length and degree of saturation of FFAs has been demonstrated (18,19). However, the mechanisms whereby chronic exposure of pancreatic β-cells to FFAs affects their action on glucose remain unclear. Recently, thiazolidinediones have been shown to prevent the impairment of islet cell function induced by fatty acids, INS-1 cells, and human islets (14,20).

Recent reports have indicated that compounds that antagonize PPAR-γ activity also display antiobesity and insulin-sensitizing effects in animals fed a high-fat diet (21). Whether these agents exert their action directly on the β-cell has not been tested.

In the present study, we evaluated the effects on insulin release and content of chronic (48 h) exposure to a pure PPAR-γ agonist and a selective PPAR-γ antagonist in a clonal rat β-cell line (INS-1e), in the presence or absence of the monounsaturated fatty acid oleate.

RESEARCH DESIGN AND METHODS

Materials. The INS-1e cell line was generously donated by Dr C.B. Wollheim (University of Geneva, Geneva, Switzerland). Tissue culture reagents were obtained from Gibco (Gibco Invitrogen, Basel, Switzerland). The PPAR-γ agonist (Ro4389679-000-001, donated by Roche, Basel, Switzerland) has a half-maximal effective concentration (EC_{50}) of 9 nmol/l in binding affinity assays. The PPAR-γ antagonist [SR202, dimethyl α-(dimethoxyphosphinyl)-*p*-chlorobenzyl phosphate] was synthetized at Ilex onc., Geneva, Switzerland (21).

Cell culture and incubation. The clonal INS-1e cells derived and selected from the parental rat insulinoma INS-1 cell line were grown in monolayer culture in RPMI-1640 medium containing 11.1 mmol/l glucose. The culture medium was supplemented with 10% heat-inactivated fetal calf serum, 1 mmol/l sodium pyruvate, 10 mmol/l HEPES, and 2 mmol/l glutamine, 50 μmol/l β-mercaptoethanol, 100 units/ml penicillin, and 100 μg/ml streptomycin. The cells were cultured at 37°C in a humidified 95% air, 5% CO_2 atmosphere. Cells were seeded in wells at a density of 1.5×10^5 cells per culture well at least 96 h before use in the insulin secretion experiments.

Insulin secretion experiments. GSIS was tested in INS-1e cells between passages 50 and 95. Forty-eight hours before the experiment, when they had

From the Department of Internal Medicine and C.N.R. Institute of Clinical Physiology, University of Pisa School of Medicine, Pisa, Italy.

Address correspondence and reprint requests to Ele Ferrannini, MD, Department of Internal Medicine, Via Roma, 67, 56100, Pisa, Italy. E-mail: ferranni@ifc.cnr.it.

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FFA, free fatty acid; GSIS, glucose-stimulated insulin secretion; KRBH, Krebs-Ringer bicarbonate HEPES buffer; PPAR, peroxisome proliferator–activated receptor.

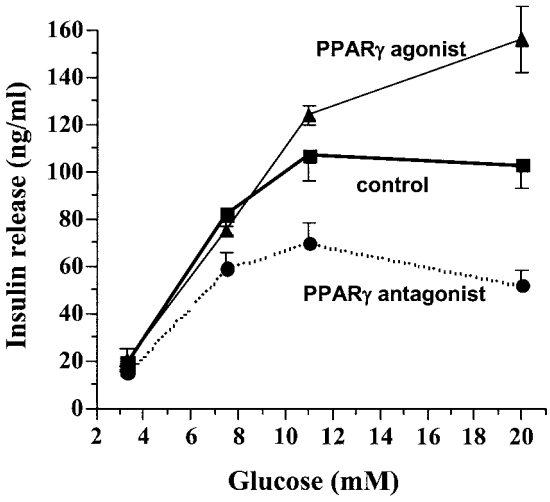

FIG. 1. Glucose-dependent increase in insulin release (i.e., insulin concentration in the supernatant) following 48-h preincubation with 11 mmol/l glucose alone (control), 11 mmol/l glucose plus 0.4 μmol/l Ro4389679-000-001 (PPAR-γ agonist), and 11 mmol/l glucose plus 0.4 mmol/l SR202 (PPAR-γ antagonist). See Tables 1 and 2 for statistical analysis of the plotted data.

reached ~80% confluence, cells were incubated in fresh RPMI-1640 medium containing 11 mmol/l glucose in the presence or absence of *1*) two concentrations of the PPAR-γ agonist (0.2 and 0.4 μmol/l) dissolved in dimethylsulfoxide (DMSO) (at a final concentration in the medium of 0.1%); *2*) two concentrations of the PPAR-γ-antagonist, SR202 (0.2 and 0.4 mmol/l in 0.1% DMSO); *3*) 1 mmol/l oleate in 2% BSA; *4*) 1 mmol/l oleate plus 0.4 μmol/l PPAR-γ agonist; and *5*) 1 mmol/l oleate plus 0.4 mmol/l SR202. Before experiments, cells were maintained for 1 h in glucose-free culture medium. Cells were then washed twice and preincubated at 37°C for 1 h in 1 ml glucose-free Krebs-Ringer bicarbonate HEPES buffer (KRBH) containing (in mmol/l) 135 NaCl, 3.6 KCl, 5 $NaHCO_3$, 0.5 Na H_2PO_4, 0.5 $MgCl_2$, 1.5 $CaCl_2$, 10 HEPES, and 0.1% BSA 0.1% (glucose free and FFA free), pH 7.4. Cells were then washed once with glucose-free KRBH and subsequently incubated for 60 min with KRBH containing 3.3, 7.5, 11.0, or 20.0 mmol/l glucose. The supernatants were collected for insulin determination, and cellular insulin contents were determined from acid-ethanol extracts. Insulin was measured by rat insulin radioimmunoassay (Linco Research, St. Charles, MO).

Statistical analysis. Data are presented as means ± SEM. Group differences were analyzed by two-way ANOVA, with medium glucose concentration (3.3, 7.5, 11, or 20 mmol/l) and preincubation conditions (*1* through *5* above) as the two main factors. In each run, the interaction term was always calculated, and individual group differences were tested by the post hoc Bonferroni-Dunn test. Insulin concentration in the supernatant (termed insulin release), insulin content, and their ratio were analyzed separately. A P value <0.05 was considered statistically significant.

RESULTS

In control incubations, glucose elicited a sharp increment in insulin release, which appeared to plateau between 11 and 20 mmol/l, at a value about fivefold higher than the value at 3.3 mmol/l (Fig. 1 and Table 1). In contrast, acute glucose stimulation had no effect on cell insulin content; therefore, the ratio of insulin release to insulin content showed a similar glucose dependence to that of insulin release itself.

Preincubation with two concentrations of the PPAR-γ agonist stimulated insulin release with no effect on insulin content; the ratio of insulin release to content was stimulated only at the highest glucose concentration ($P < 0.01$) (Table 1).

The PPAR-γ antagonist was ineffective at the lower concentration, but markedly depressed insulin release and the insulin ratio at the higher concentration, without changing cellular insulin content (Fig. 1 and Table 2).

TABLE 1
Effect of PPAR-γ activation

Preincubation (48 h)	Insulin in medium (ng/ml)					Insulin content (μg/ml)					Ratio				
	3.3	7.5	11	20	P	3.3	7.5	11	20	P	3.3	7.5	11	20	P
[11 mmol/l]G	19 ± 4	82 ± 5	107 ± 11	103 ± 10		2.0 ± 0.1	1.8 ± 0.2	1.9 ± 0.1	1.8 ± 0.1		1.0 ± 0.2	4.4 ± 0.3	5.3 ± 0.6	5.7 ± 0.7	
[11 mmol/l]G + [0.2 μmol/l]γ	20 ± 5	89 ± 2	113 ± 8	154 ± 14	*	1.9 ± 0.1	2.1 ± 0.1	2.0 ± 0.1	1.9 ± 0.1	NS	1.0 ± 0.3	4.0 ± 0.1	5.3 ± 0.5	7.6 ± 0.9	NS
[11 mmol/l]G + [0.4 μmol/l]γ	20 ± 5	75 ± 2	124 ± 4	156 ± 14	*	2.1 ± 0.1	2.1 ± 0.2	2.0 ± 0.1	2.0 ± 0.1	NS	0.9 ± 0.3	3.6 ± 0.2	6.0 ± 0.4	7.3 ± 0.7	NS

*$P < 0.01$ vs. [11 mmol/l]G; $n = 33$ in each row. [11 mmol/l]G, 11 mmol/l glucose; [0.2 μmol/l]γ, 0.2 μmol Ro4389679-000-001 (PPAR-γ agonist); [0.4 μmol/l]γ, 0.4 μmol/l Ro4389679-000-001 (PPAR-γ agonist).

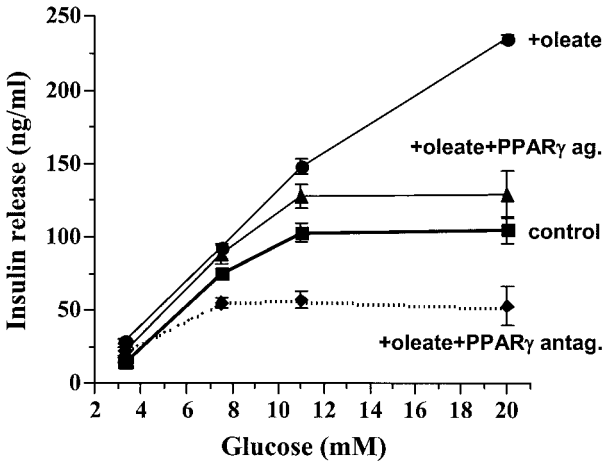

FIG. 2. Glucose-dependent increase in insulin release (i.e., insulin concentration in the supernatant) following 48-h preincubation with 11 mmol/l glucose alone (control), 11 mmol/l glucose alone plus 1 mmol/l oleate (oleate), 11 mmol/l glucose plus oleate plus 0.4 μmol/l Ro4389679-000-001 (PPAR-γ agonist), and 11 mmol/l glucose plus oleate plus 0.4 mmol/l SR202 (PPAR-γ antagonist). See Table 3 for statistical analysis of the plotted data.

Oleate was strongly stimulatory of insulin release (Fig. 2) and significantly increased insulin content; this effect was blunted by the highest concentration of the PPAR-γ agonist and was fully reversed by the higher concentration of the PPAR-γ antagonist (Table 3).

Glucose dependence of insulin release was preserved under all preincubation conditions ($P < 0.0001$).

DISCUSSION

The main finding of the current study is that glucose-stimulated insulin release by INS-1e cells can be modulated symmetrically by PPAR-γ agonism and PPAR-γ antagonism. Oleate, on the other hand, potentiates GSIS, but this effect is abolished by both PPAR-γ agonism and PPAR-γ antagonism.

While there appears to be consensus that β-cells do express PPAR-γ, direct exposure of β-cells to agonists of these receptors have yielded conflicting results. Thus, high concentrations of troglitazone (>10 μmol/l) suppressed GSIS in INS-1 cells, as reported in other pancreatic β-cell lines (8–10). In a recent study, treatment with troglitazone (5 μmol/l) significantly suppressed insulin secretion at a low glucose concentration of 2.8 mmol/l, while it caused significant enhancement of GSIS at concentrations of 5.6 and 11.1 mmol/l glucose (20). These data were interpreted as evidence that enhancement of GSIS depends on modulation by troglitazone, possibly through PPAR-γ (13,20). Inoue et al. (22) reported that troglitazone increased insulin content in β-cells in 90% pancreatectomized rats and in subjects with type 2 diabetes. Prigeon et al. (23) reported that troglitazone treatment lowered the ratio of proinsulin to immunoreactive insulin, thereby implying that troglitazone may have direct effects on β-cell function. We used concentrations of Ro4389679-000-001 (which has an EC_{50} similar to that of rosiglitazone) that fall into the range of serum concentration measured in patients with type 2 diabetes during chronic treatment with rosiglitazone (24). The insulin stimulatory effect was clearly evident at 20 mmol/l glucose and quantitatively modest.

TABLE 2
Effect of PPARγ antagonism

Preincubation (48 h)	Insulin in medium (ng/ml)					Insulin content (μg/ml)					Ratio				
	3.3	7.5	11	20	*P*	3.3	7.5	11	20	*P*	3.3	7.5	11	20	*P*
[11 mmol/l]G	13 ± 1	65 ± 4	97 ± 12	84 ± 6		2.8 ± 0.2	2.4 ± 0.2	2.5 ± 0.2	2.7 ± 0.3		0.5 ± 0.1	2.7 ± 0.3	3.8 ± 0.6	3.1 ± 0.2	
[11 mmol/l]G + [0.2 mmol/l]SR	12 ± 1	77 ± 7	109 ± 20	84 ± 15	NS	2.8 ± 0.3	2.7 ± 0.2	2.7 ± 0.3	2.5 ± 0.3	NS	0.4 ± 0.1	2.7 ± 0.1	3.7 ± 0.3	3.3 ± 0.4	NS
[11 mmol/l]G + [0.4 mmol/l]SR	15 ± 1	59 ± 7	70 ± 8	51 ± 6	*	2.6 ± 0.2	2.3 ± 0.2	2.5 ± 0.1	2.7 ± 0.2	NS	0.6 ± 0.1	2.6 ± 0.3	2.7 ± 0.2	1.8 ± 0.2	†

*$P < 0.002$ and †$P < 0.01$ vs. [11 mmol/l]G; n = 21, 24, and 36 for the three rows. [11 mmol/l]G, 11 mmol/l glucose; [0.2 mmol/l]SR, 0.2 mmol/l SR202 (PPAR-γ antagonist; [0.4 mmol/l]SR, 0.4 mmol/l SR202 (PPAR-γ antagonist).

TABLE 3
Effect of oleate, alone or with PPAR-γ agonist or PPAR-γ antagonist*

Preincubation (48 h)	Insulin in medium (ng/ml)					Insulin content (μg/ml)					Ratio				
	3.3	7.5	11	20	*P*	3.3	7.5	11	20	*P*	3.3	7.5	11	20	*P*
[11 mmol/l]G	15 ± 1	75 ± 3	103 ± 6	105 ± 9		2.6 ± 0.1	2.3 ± 0.1	2.3 ± 0.1	2.5 ± 0.1		0.6 ± 0.1	3.1 ± 0.2	4.3 ± 0.3	4.1 ± 0.4	
[11 mmol/l]G + ole	29 ± 3	93 ± 6	148 ± 8	235 ± 16	†	3.0 ± 0.1	2.7 ± 0.1	2.6 ± 0.1	2.8 ± 0.2	†	0.9 ± 0.2	3.3 ± 0.2	5.4 ± 0.4	7.8 ± 0.3	†
[11 mmol/l]G + ole + [0.4 μmol/l]γ	22 ± 3	88 ± 4	128 ± 6	129 ± 13	‡§	2.7 ± 0.2	2.5 ± 0.1	2.8 ± 0.2	3.0 ± 0.2	‡	0.8 ± 0.1	3.4 ± 0.2	4.3 ± 0.1	4.1 ± 0.4	§
[11 mmol/l]G + ole + [0.4 mmol/l]SR	22 ± 2	55 ± 3	57 ± 5	53 ± 3	§†¶	2.6 ± 0.2	2.5 ± 0.2	2.5 ± 0.2	2.5 ± 0.2	NS	0.8 ± 0.1	2.3 ± 0.3	2.4 ± 0.4	2.1 ± 0.2	§†¶

*n = 83, 27, 24, and 36 for each of the four rows. †$P < 0.0001$ and ‡$P < 0.001$ vs. [11 mmol/l]G; §$P < 0.0001$ vs. [11 mmol/l]G + ole; ¶$P < 0.0001$ vs. [11 mmol/l]G + ole + [0.4 μmol/l]γ. [11 mmol/l]G, 11 mmol/l glucose; ole, 1 mmol/l oleate; [0.4 μmol/l]γ, 0.4 μmol/l Ro43896679-000-001 (PPAR-γ agonist); [0.4 mmol/l]SR, 0.4 mmol/l SR202(PPAR-γ antagonist).

The synthetic compound SR202, belonging to the phosphonophosphate family, specifically inhibits PPAR-γ transcriptional activity (21). Preincubation of INS-1e cells with SR202 markedly depressed GSIS without affecting insulin content (Table 2). This finding indicates that GSIS normally requires PPAR-γ activity; depression of such tonic activity results in impaired GSIS.

FFAs may be associated with lipotoxicity (15–17) in β-cells. The precise mechanisms whereby chronic exposure of β-cells to FFA affects their function remain unclear, and different results have been obtained in relation to the chain length and degree of saturation of FFA (18,19). In our hands, 1 mmol/l oleate potentiated GSIS, and significantly increased insulin content in INS-1e cells, suggesting an effect on both insulin biosynthesis and release. Kliewer et al. (25) have shown that certain mono- and polyunsaturated fatty acids (including oleate) bind directly to PPAR-γ at physiological concentrations, providing evidence that, in addition to PPAR-α, PPAR-γ serves as a physiological sensor of lipid levels. Furthermore, thiazolidinediones have been shown to prevent the impairment of islet cell function induced by fatty acids in INS-1 cells and human islets (14,20). In our preincubation studies, both stimulatory concentrations of the PPAR-γ agonist and inhibitory concentrations of the PPAR-γ antagonist abolished the potentiating effect of oleate. Interestingly, however, the inhibition by the antagonist was comparable to that achieved in the absence of oleate, while the effect of the agonist reproduced the stimulation seen in the absence of oleate (Fig. 2). In other words, the agonist blocked the action of oleate while retaining its own (Table 3). These results are best explained by competitive binding of the agonist and oleate at the level of the receptor: when this is occupied by stimulating concentrations of the agonist, oleate is without effect. On the other hand, barring the pathway downstream to the binding step, namely at the level of coactivator recruitment with the antagonist, renders stimulation by either oleate or the agonist ineffective, and depresses baseline receptor activity.

In conclusion, INS-1e cells are subject to symmetrical modulation of GSIS by PPAR-γ activity. Further studies are required to gain insight into the fine intracellular changes underlying the observed modulation of function as well as the relevance of such phenomenon to in vivo control of glucose and lipid homeostasis.

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