Insulin-sparing effects of troglitazone in rat pancreatic islets

L C Bollheimer, S Troll, H Landauer, C E Wrede, J Schölmerich and R Buettner
Department of Internal Medicine I, University of Regensburg, D-93042 Regensburg, Germany

(Requests for offprints should be addressed to R Buettner; Email: roland.buettner@klinik.uni-regensburg.de)

Abstract

Thiazolidinediones (TZDs) have been suggested to act beneficially on pancreatic islet function and on beta-cell viability but data concerning direct effects on isolated islets are controversial. Therefore, we have examined parameters of pancreatic insulin and glucagon secretion and biosynthesis in TZD-exposed rat pancreatic islets under physiological glucose level conditions and under conditions of glucolipotoxicity.

Primary rat islets were incubated for 2.5 h with or without troglitazone (10 µM) in 5.6 mM glucose (standard glucose levels) and 16.7 mM glucose (high glucose levels); a subgroup was additionally treated with oleate (200 µM) to simulate acute glucolipotoxicity. Insulin and glucagon secretion, intracellular content and their respective mRNAs were quantified. Newly synthesized insulin was determined by pulse-labeling experiments.

Troglitazone reduced insulin secretion at standard and high glucose levels by about one-third ($P \leq 0.05$). Insulin content was decreased at 5.6 mM glucose but increased at 16.7 mM glucose by the presence of troglitazone ($P \leq 0.05$). Newly synthesized insulin mRNA and preproinsulin mRNA decreased by about 20% at standard glucose levels ($P \leq 0.05$). Glucagon secretion was augmented by troglitazone in islets under high glucose conditions by an additional 50% ($P \leq 0.05$). No clear beneficial troglitazone effects were observed under glucolipotoxic conditions.

The reduced insulin secretion and biosynthesis at standard glucose levels can be interpreted as an insulin-sparing effect. Troglitazone effects were less pronounced at high glucose alone or in combination with oleate. From a clinical point of view, these results indicate a greater benefit of troglitazone for beta-cell function in hyperinsulinemic, but normoglycemic patients with insulin resistance or early type 2 diabetes without major insulin secretion deficits and/or pronounced hyperglycemia.

Journal of Molecular Endocrinology (2003) 31, 61–69

Introduction

Thiazolidinediones (TZDs) are widely used for the treatment of type 2 diabetes (Olefsky & Saltiel 2000, Parker 2002). As ligands of the nuclear peroxisome proliferator-activated receptor-γ (PPARγ) they mainly affect cellular gene expression leading to an improved peripheral tissue sensitivity (Reginato & Lazar 1999). As PPARγ is highly expressed in islets of Langerhans (Dubois et al. 2000), direct antidiabetic effects of TZDs on the pancreatic beta-cell are also under discussion. By using hyperglycemic and hyperlipidemic (fa/fa) Zucker (ZDF) rats, Unger and coworkers suggested a protective lipogenic effect of the TZD compound troglitazone against fatty acid-induced loss of beta-cell function and cell death (Shimabukuro et al. 1998, Higa et al. 1999). While these anti-lipotoxic effects of troglitazone were supported by an in vitro study with fatty acid-exposed INS-1 cells (Kawai et al. 2002), the findings are challenged by controversial results employing isolated primary islets (Cnop et al. 2002). In clinical studies it has been shown that insulin levels decrease during TZD treatment (Prigeon et al. 1998, Buchanan et al. 2000), and the question remains whether this is due solely to the reduction of peripheral insulin resistance or at least partially caused by a specific TZD effect on pancreatic islet function. In rat pancreatic islets, Masuda et al. (1995) described a dose-dependent effect of troglitazone with a potentiation of insulin secretion at doses of
1–10 µM and a diminution at doses of 100 µM. In the pharmacologically relevant range of 5–10 µM troglitazone, we and others have observed only marginal changes in insulin release when using the clonal beta-cell lines HIT or INS-1 (Ohtani et al. 1998, Bollheimer et al. 2002, Kawai et al. 2002).

Taken together, data concerning immediate effects of TZDs (such as troglitazone) on rat pancreatic islets are still sparse and inconclusive. Although previous studies hint at a direct effect of TZDs on the pancreatic beta-cell of normal and free fatty acid (FFA)-exposed islets – which might partly contribute to their clinical action – this aspect of TZD pharmacodynamics has not been well elucidated. In the present study we characterize the beta-cell insulin secretion and biosynthesis as well as the alpha-cell glucagon metabolism in TZD-exposed rat pancreatic islets under standard glucose (5·6 mM) as well as high glucose (16·7 mM) and high oleate levels (200 µM pre-complexed to 0·2% w/v BSA) to simulate normoglycemic, hyperglycemic and glucolipotoxic – i.e. hyperglycemia combined with hyperlipacidemia – conditions. We have chosen troglitazone as a model TZD because it was the first clinically approved drug of its class with the largest background of clinical, physiological and molecular pharmacodynamic data at hand (Parker 2002).

Materials and methods

Materials

RIAs for immunoreactive insulin (IRI) and C-peptide as well as pancreatic glucagon (IRG) were performed using respective kits from Linco Research Inc. (St Louis, MO, USA). L-[35S]methionine (43·5 TBq/mmol) was from NEN (Boston, MA, USA). Pansorbin was purchased from Calbiochem (La Jolla, CA, USA). Fatty acid ultra-free BSA (fraction V), protease inhibitor cocktail (Complete), Collagenase P (1·5 U/mg), as well as all technical and chemical equipment for semiquantitative LightCycler PCR were from Roche Diagnostics (Mannheim, Germany). Reverse transcription was performed employing a kit from Promega (Madison, WI, USA). Cellulose membrane filters (MF-Millipore, 0·45 µm) and a sample manifold apparatus were from Millipore (Eschborn, Germany). All other analytical grade biochemicals were purchased from Sigma-Aldrich (Taufkirchen, Germany) or from Merck Eurolab (Darmstadt, Germany). Troglitazone (5-(4-(6-hydroxy-2,5,7,8-tetra-methylchroman-2-ylmethoxy)benzyl)-2,4-thiazolidine-dione) was kindly provided by Sankyo (Tokyo, Japan).

Islet isolation and culture

Pancreatic islets were isolated from 200 g male Wistar rats by collagenase digestion and Histopaque–Ficoll density gradient centrifugation. The newly isolated islets were maintained for 16 h in RPMI-1640/11·2 mM glucose/10% w/v fetal bovine serum at 37 °C in a 5% CO2 atmosphere (Bollheimer et al. 1998). Afterwards islets were employed for the experiments as described below. All experimental animals were housed, handled and used in accordance with the local animal rights committee’s guidelines.

Concentration of troglitazone and oleate

Troglitazone was used in a standard concentration of 10 µM with dimethylsulfoxide (DMSO) as vehicle for solubilization [cfinal[DMSO]=0·01% v/v] (Bollheimer et al. 2002, Cnop et al. 2002, Kawai et al. 2002). The latter was added at the same concentration to the non-troglitazone-containing samples. Oleic acid (cis Δ-9 octadecenoic acid) was used in a total concentration of 200 µM pre-complexed to 0·2% w/v fatty acid ultra-free BSA (fraction V), as previously described (Wrede et al. 2002).

Analysis of secretion and content of IRI, C-peptide and glucagon

Groups of 35 islets were washed and transferred into 300 µl Krebs–Ringer bicarbonate buffer/16 mM Hepes (pH 7·4) at 5·6 mM glucose. After a 2-h pre-incubation islets were incubated for further 150 min at either 5·6 or 16·7 mM glucose in the presence or absence of 10 µM troglitazone. The troglitazone exposure at 16·7 mM glucose was also performed in the additional presence of 200 µM oleate. At the end, the supernatants (300 µl) were collected, centrifuged and kept frozen pending analysis by RIA for IRI, C-peptide and glucagon. The islets themselves were washed in PBS and then lysed in 300 µl detergent-containing buffer (50 mM Hepes (pH 8·0), 0·1% v/v Triton X-100 plus
protease inhibitor cocktail). After sonication and centrifugation at 10 000 g for 2 min to remove debris (Bollheimer et al. 1998), aliquots were taken for assessment of (i) lysate protein (bicinchoninic method) and (ii) the corresponding contents of IRI, C-peptide and glucagon.

Analysis of total protein and proinsulin biosynthesis

To each sample, L-[35S]-methionine (0.2 nmol/1.85 MBq) was added 30 min prior to the end of exposure. To measure the rate of total protein biosynthesis, aliquots of the insular lysates were treated with 10% w/v trichloroacetic acid (TCA) to precipitate cellular protein. The precipitates were then trapped onto MF-Millipore membranes and extensively washed in 5% w/v TCA by flow through on a sample manifold apparatus prior to analysis for incorporated 35S by a standard scintillation counting procedure (Itoh & Okamoto 1980). To assess the rate of proinsulin biosynthesis, aliquots of the insular lysates were subjected to immunoprecipitation against IRI (Skelly et al. 1998). After additional chemical precipitation by TCA, IRI was spotted onto MF-Millipore membranes and analyzed for the amount of incorporated 35S.

LightCycler analysis of preproinsulin (PPI) mRNA and preproglucagon (PPG) mRNA content

Batches of ten islets were incubated for 150 min under the same conditions as described above. Afterwards cells were lysed and cytoplasmic RNA was prepared according to the method of Gough (1988). First-strand complementary DNA was synthesized by priming with arbitrary hexamers. Subsequent PCR amplification and analysis were achieved in 20 µl glass capillaries using the Roche LightCycler system with the respective software (version 3.5) and a master mix containing Taq DNA polymerase, SYBR-green I and the deoxyribonucleotides (Lekanne Deprez et al. 2002). The following primer pairs were employed in a final concentration of 1 µM for (i) analysis of PPI mRNA: 5'-TGTGGTTCTCACTTTGGGAG-3' (sense, 91–110) and 5'-ATGCTGGTGACACGAC TGAT-3' (antisense, 278–296); (ii) analysis of PPG mRNA: 5'-TCGTGCGCTGGATTTTGGTA-3' (sense, 17–36) and 5'-CAATGGTGTTCGGGT TCCTG-3' (antisense, 246–265); (iii) analysis of glyceraldehyde-3-phosphate-dehydrogenase mRNA (GAPDH): 5'-TTAGCACCCTGCGCCAAGC-3' (sense, 495–511) and 5'-CTTACTCCTTTGGAGG CCATG-3' (antisense, 1014–1033). For each primer combination optimal MgCl2 concentration and annealing temperature were experimentally determined. For PPI and PPG a MgCl2 concentration of 5 mM and an annealing temperature of 57 °C resulted in efficiencies (E) of 1.89 and 1.97 respectively. For GAPDH optimal conditions were given by 3 mM MgCl2 and 56 °C annealing temperature (E_{GAPDH}=1.96). The first-strand DNA-samples were amplified for 40 cycles of denaturation (95 °C for 5 s), annealing (56 or 57 °C for 5 s) and extension (72 °C for 22 s). The initial amount of the cDNA content in each sample (N_{sample}) was then semiquantitatively assessed by comparing the experimentally determined crossing point (CP_{sample}) with crossing points from subsequent dilutions of a standardized pooled islet cDNA (CP_{standard}) and the respective concentrations (N_{standard}) according to: (N_{sample} \div N_{standard}) = (E_{CP_{standard}} \div E_{CP_{sample}}).

After completion of PCR amplification, melting curve analysis (Ririe et al. 1997) was routinely performed for each sample to confirm primer specificity.

Statistical analysis

Data are presented as means ± S.E. of at least five independent experiments. After verification of normal distribution by the Kolmogorov–Smirnov test, statistically significant differences were analyzed using unpaired Student’s t-test where P<0.05 was considered significant.

Results

Troglitazone effects on the secretory output and cellular content of IRI mainly at physiological glucose levels

Pancreatic rat islets were cultured for 2.5 h with or without 10 µM troglitazone at standard glucose (5.6 mM glucose) and high glucose (16.7 mM glucose) levels. Cumulative release of IRI was determined from the cellular supernatants, whereas the final cellular IRI content was measured from the islet lysates. The results are given in detail in
Table 1 Effects of troglitazone on insulin and glucagon secretion and content. Rat pancreatic islets were incubated with or without troglitazone (10 μM) at standard glucose (5·6 mM, ST−/ST+) and high glucose (16·7 mM, HT−/HT+) conditions for 2·5 h. Immunoreactive insulin and pancreatic glucagon were measured in supernatants and islet lysates by specific RIA. The results are corrected for equal amounts of islet lysate protein and are depicted as means±S.E. of six or seven independent experiments.

<table>
<thead>
<tr>
<th></th>
<th>ST−</th>
<th>ST+</th>
<th>HT−</th>
<th>HT+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cumulative secretion (pmol/mg protein)</td>
<td>68±18</td>
<td>42±6*</td>
<td>138±22</td>
<td>98±17*</td>
</tr>
<tr>
<td>Content (nmol/mg protein)</td>
<td>12±0.8</td>
<td>10.2±1.0*</td>
<td>9.9±1.2</td>
<td>12.6±1.3*</td>
</tr>
<tr>
<td>Secretion:content (%)</td>
<td>0.53±0.13</td>
<td>0.46±0.09</td>
<td>1.44±0.17</td>
<td>0.79±0.15*</td>
</tr>
</tbody>
</table>

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cumulative secretion (pmol/mg protein)</td>
<td>5±2</td>
<td>4±1</td>
<td>3±1</td>
<td>5±1</td>
</tr>
<tr>
<td>Content (nmol/mg protein)</td>
<td>0.7±0.1</td>
<td>0.611±0.1</td>
<td>0.6±0.1</td>
<td>0.7±0.2</td>
</tr>
<tr>
<td>Secretion:content (%)</td>
<td>0.63±0.10</td>
<td>0.77±0.13</td>
<td>0.6±0.10</td>
<td>0.69±0.10</td>
</tr>
</tbody>
</table>

*P<0·05 compared with untreated controls at equal glucose levels.

Table 1. Untreated islets maintained at standard glucose levels (ST−) released 68±18 pmol insulin/mg lysate protein into the supernatant. Addition of troglitazone (ST+) significantly reduced insulin secretion by 38±9% (P≤0·01). Incubation of islets at high glucose conditions without troglitazone (HT−) doubled insulin secretion when compared with islets at standard glucose (P≤0·01). In the presence of troglitazone (HT+), insulin secretion was blunted also under high glucose conditions, although this effect was not as pronounced as at standard glucose levels (29±12% reduction compared with control, P≤0·05). The stimulating effect of glucose on insulin secretion was not influenced by troglitazone treatment (IRI secretion of HT− = 202±32% of ST−, IRI secretion of HT+ = 233±40% of ST+, P=0·29, when comparing the IRI increments with and without troglitazone addition).

In ST− islets, islet content of IRI was 12±1 nmol/mg lysate protein (Table 1). Addition of troglitazone decreased islet insulin content by 15±8% (P≤0·05). High glucose levels per se led to an average decline of 20% in intracellular IRI when compared with ST− (P≤0·05). Addition of troglitazone at high glucose conditions elevated the final IRI content by 27±13% when compared with HT− (P≤0·05).

The conversion rate of proinsulin to insulin was assessed by relating corresponding C-peptide and IRI levels in the cellular supernatants as previously described (Bollheimer et al. 2001). The ratio between C-peptide and IRI did not differ significantly between ST− (0·39±0·05) and ST+ (0·47±0·07, P=0·30), nor between HT− (0·66±0·11) and HT+ (0·46±0·08, P=0·12). Thus, we found no clear evidence for troglitazone-induced changes of posttranslational proinsulin processing at both standard and high glucose conditions.

Troglitazone effects on the secretory output and cellular content of pancreatic glucagon

The same islets used for the experiments described above were also analyzed for pancreatic glucagon. The results are included in Table 1. ST− islets released 5±2 pmol glucagon/mg lysate protein and contained 0·7±0·1 nmol glucagon/mg lysate protein. Troglitazone exposure at 5·6 mM glucose did not significantly alter the examined parameters of islet alpha-cell function. Looking at high glucose conditions, glucagon secretion in HT− islets was reduced by 32±16% (P≤0·05) compared with the standard glucose ST− controls without major changes in glucagon content. Here, the presence of troglitazone (HT+) led to an augmented release of glucagon by an additional 50% (P≤0·05) without affecting intracellular glucagon content.

Molar ratios of insulin to glucagon

Assessing glucagon and insulin secretion in terms of molar quantities, we saw about a 15-fold higher secretion of insulin over glucagon in the ST− group. Due to the decrease in insulin secretion after
troglitazone incubation this ratio was lowered by about one-third in the \( ST^+ \) group, but this result did not reach statistical significance due to inter-experimental variability. In \( HT^- \) islets the insulin-to-glucagon ratio was approximately 3-fold higher than in \( ST^- \) islets (\( HT^- \) 49 ± 12:1 vs \( ST^- \) 15 ± 4:1, \( P \leq 0\cdot05 \)). Additional troglitazone treatment (\( HT^+ \)) halved this ratio (\( P \leq 0\cdot05 \)).

Looking at molar quantities of intracellular IRI and IRG content, we found about 20 times more insulin than glucagon in cellular lysates of \( ST^- \) islets. In this regard there were no significant changes in \( ST^+ \), \( HT^- \) and \( HT^+ \).

### Newly synthesized total protein and IRI in troglitazone-treated rat islets

\(^{35}\)S incorporation during the last 30 min of the 2.5 h incubation period served as a measure of total protein biosynthesis (Table 2). Compared with \( ST^- \), troglitazone exposure at 5-6 mM glucose (\( ST^+ \)) did not lead to major changes in protein synthesis. At high glucose levels (\( HT^- \)) the protein synthesis rate was increased by an additional 30% in comparison with \( ST^- \). The presence of troglitazone (\( HT^+ \)) generated no additional effect over \( HT^- \).

By correcting \(^{35}\)S incorporation into IRI immunoprecipitates with \(^{35}\)S incorporation into total lysate protein, it is possible to bring out specific effects on the de novo biosynthesis of proinsulin that are above those on general protein biosynthesis (Itoh & Okamoto 1980, Bollheimer et al. 1998). Specific IRI biosynthesis decreased by about one-fifth after additional troglitazone exposure at standard glucose levels (\( P \leq 0\cdot05 \)). After a 2.5 h period at high glucose levels, specific IRI synthesis in troglitazone-treated \( HT^+ \) islets was about one-third lower than in \( ST^- \) islets (\( P \leq 0\cdot05 \)), but no distinct effect of troglitazone could be observed when compared with untreated islets (\( HT^- \)) at high glucose levels.

### Table 2 Effects of troglitazone on insulin biosynthesis.

Rat pancreatic islets were incubated with or without troglitazone (10 \( \mu \)M) at standard glucose (5-6 mM, \( ST^-/ST^+ \)) and high glucose (16-7 mM, \( HT^-/HT^+ \)) conditions together with \(^{35}\)S methionine. Newly synthesized protein was calculated from the incorporation of radioactivity into TCA precipitates, newly synthesized proinsulin from incorporation into insulin immunoprecipitates. The results are corrected for total lysate protein and shown as the mean change from control (\( ST^- \)) as percent ± S.E. of six independent experiments.

<table>
<thead>
<tr>
<th></th>
<th>( ST^- )</th>
<th>( ST^+ )</th>
<th>( HT^- )</th>
<th>( HT^+ )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newly synthesized total protein</td>
<td>100±0</td>
<td>91±15</td>
<td>136±17*</td>
<td>131±20</td>
</tr>
<tr>
<td>Newly synthesized insulin</td>
<td>100±0</td>
<td>62±9*</td>
<td>95±13</td>
<td>92±11</td>
</tr>
<tr>
<td>Specific insulin biosynthesis</td>
<td>100±0</td>
<td>80±12*</td>
<td>83±8*</td>
<td>77±8*</td>
</tr>
</tbody>
</table>

*\( P \leq 0\cdot05 \) compared with control.

### Cellular content of PPI mRNA and PPG mRNA

For further investigation of biosynthetic effects, semiquantitative assessment for PPI mRNA, PPG mRNA and GAPDH mRNA as housekeeping gene were performed using a real-time PCR technique (Briaud et al. 2001). The results are detailed in Fig. 1. Compared with the \( ST^- \) group, islets which
had been additionally exposed to troglitazone at standard glucose levels showed a decrease in their cellular PPI mRNA content by approximately one-fifth (\(P_{p<0.05}\)). The PPI mRNA content at high glucose conditions (\(HT^-\)) tended to decline when compared with \(ST^-\) (0.85 ± 0.2-fold of control, \(P=0.08\)). Under high glucose conditions there was no clear independent effect of troglitazone on the cellular PPI mRNA content. Cellular PPG mRNA content of \(ST^+\) and \(HT^+\) did not significantly differ from that of their respective controls (\(P=0.07\) when comparing \(ST^+\) and \(ST^-\), \(P=0.28\) when comparing \(HT^+\) and \(HT^-\)).

**Results**

### Effects of troglitazone under conditions of glucolipotoxicity

Elevated glucose and FFA levels are hallmarks of adipogenic type 2 diabetes. To elucidate whether FFA exposure changed the effects of troglitazone in a high glucose setting (so-called ‘glucolipotoxicity’), we exposed islets to troglitazone and oleate at 16–7 mM glucose and measured the effects on insulin and glucagon content, secretion and mRNA content as well as the de novo insulin biosynthesis as described above. As shown in Table 3, troglitazone tended to impair insulin secretion, similar to the results described above. However, due to high inter-experiment variability, the resulting 20% decrease was not statistically significant. Other specific troglitazone effects on the examined parameters of beta-cell function were not detected. With respect to glucagon, we did not find any troglitazone-induced changes of glucagon secretion, content or PPG mRNA content under the condition of glucolipotoxicity.

### Discussion

TZD derivatives such as troglitazone modulate cell function in a pleiotropic manner mainly through PPARs (Reginato & Lazar 1999). Their anti-diabetic potency is thereby generally attributed to an improvement of peripheral insulin sensitivity. Nonetheless, TZDs may also directly influence the endocrine pancreas since PPARγ is expressed both in rodent and in human pancreatic islets (Dubois et al. 2000). We therefore carried out an in-depth characterization of the effects of the TZD prototype troglitazone on parameters of rat islet beta- and alpha-cell function under standard and high glucose (5.6/16.7 mM) as well as glucolipotoxic (16.7 mM glucose and 200 µM oleate pre-complexed to 0.2% BSA) conditions.

### Islet hormone secretion

We observed a drop in insulin secretion of about one-third after a 2.5 h incubation of rat pancreatic islets with 10 µM troglitazone at both standard and high glucose levels. Notably, the relative stimulation of insulin secretion by high glucose was not influenced by troglitazone treatment.

Such an insulin-sparing effect of troglitazone on beta-cell insulin output has not been described before. In a previous in vitro study looking at troglitazone effects on normal primary rat islets, Masuda et al. (1995) reported an increased insulin secretion as a response to troglitazone (1–10 µM) after 30 min of incubation at 11.2 mM glucose, which reflects a first phase secretory response of the pancreatic beta-cell. It has to be noted that the same authors reported a troglitazone-induced decrease in rapid insulin secretion in islets at higher troglitazone concentrations (\(c_{troglitazone}=100\ \mu M\)) as well as in the clonal beta-cell line HIT-T15 (\(c_{troglitazone}=10–100\ \mu M\)) (Masuda et al. 1995).

---

**Table 3** Effects of troglitazone on parameters of islet function under conditions of glucolipotoxicity. After preconditioning at 11.2 mM glucose, pancreatic islets were incubated at 16.7 mM glucose and 200 µM oleate to simulate glucolipotoxic conditions. The effect of 10 µM troglitazone on protein content, islet insulin and glucagon content, secretion and mRNA expression as well as specific insulin biosynthesis are shown as relative change (%) from the control (i.e. glucose 16.7 mM, no addition of oleate or troglitazone). The data represent means±S.E. of six or seven independent experiments.

<table>
<thead>
<tr>
<th>Troglitazone (µM)</th>
<th>0</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Content</td>
<td>92±7</td>
<td>114±13*</td>
</tr>
<tr>
<td>Secretion</td>
<td>96±32</td>
<td>76±27</td>
</tr>
<tr>
<td>mRNA content</td>
<td>90±14</td>
<td>107±29</td>
</tr>
<tr>
<td>Glucagon</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Content</td>
<td>118±19</td>
<td>117±13</td>
</tr>
<tr>
<td>Secretion</td>
<td>155±38</td>
<td>161±37</td>
</tr>
<tr>
<td>mRNA content</td>
<td>103±18</td>
<td>115±24</td>
</tr>
</tbody>
</table>

*\(P<0.05\) compared with islets not treated with troglitazone.
In agreement with our results, Shimabukuro et al. (1998) report a moderate, albeit not statistically significant reduction of insulin secretion after a 48 h incubation with 10 µM troglitazone in lean ZDF (+/+) rats. In the clonal beta-cell line INS-1, a stable to a decreasing insulin release has been reported after prolonged (4 h) as well as chronic (48 h) exposure to 10 µM troglitazone (Bollheimer et al. 2002, Kawai et al. 2002).

The studies cited above illuminate the controversy about troglitazone-induced changes of insulin secretion in vitro. Clinical studies with animals and humans chronically treated with troglitazone consistently show a decrease in plasma insulin levels (Prigeon et al. 1998, Buchanan et al. 2000). This effect is commonly attributed to an increased peripheral insulin sensitivity, but it also might be partly accounted for by the reduction of insulin secretion we describe here. The fact that glucagon secretion was elevated in troglitazone-treated islets at high glucose levels rules out a general lowering, i.e. toxic, effect of troglitazone on islet hormone output at the concentration we employed. This stimulation of glucagon release rather strengthens the notion that troglitazone might acutely act in a contra-insulinar fashion.

Islet hormone synthesis

To discern whether the decrease in insulin secretion was accompanied by changes in islet hormone biosynthesis, we next examined the intracellular content of IRI and IRG and their corresponding mRNAs as well as the amount of newly synthesized PPI. To our knowledge, these aspects of troglitazone action on primary pancreatic islets have not been analyzed in detail before, thereby limiting the possibility of comparing our results with other studies (Bollheimer et al. 2002, Schinner et al. 2002).

Concerning insulin, we found a parallel decrease of both intracellular IRI content and intracellular PPI mRNA at standard glucose levels. This points to troglitazone-induced changes in PPI mRNA transcription and/or stability. Further evidence for a specific inhibition of PPI biosynthesis by troglitazone at standard glucose levels was provided from the pulse-labeling experiments showing a specific decrease of newly synthesized IRI. Non-specific causes of this impairment in insulin biosynthesis do not seem likely as the total amount of newly synthesized protein did not differ between troglitazone-treated islets and controls.

Under high glucose conditions, IRI content was elevated by troglitazone. This could be due to the aforementioned sparing effect in insulin secretion, particularly as there was no significant change in pulse label-quantified PPI biosynthesis or mRNA content in comparison with the corresponding controls. This is in accord with our previous study with INS-1 cells, where troglitazone per se did not affect parameters of PPI transcription and translation at high glucose levels ($c_{\text{glucose}} = 11.2 \text{ mM}$) (Bollheimer et al. 2002). Principally, we cannot exclude other mechanisms, e.g. a diminished intracellular insulin degradation which might further contribute to the increased intracellular insulin content after troglitazone exposure under high glucose conditions (Halban & Wollheim 1980, Bollheimer et al. 2001).

Concerning glucagon, neither troglitazone nor high glucose induced significant changes in the intracellular content. PPG mRNA levels tended to decrease after troglitazone treatment at both glucose levels employed. A recent study by Schinner et al. (2002) has confirmed a similar impairment of PPG mRNA synthesis in primary islets after TZD treatment, although a strict comparison with our results is not possible due to (i) the TZD employed, namely rosiglitazone, which can activate PPARγ more effectively than troglitazone (Camp et al. 2000), (ii) a longer incubation time of 48 h, and (iii) the fact that mouse and not rat islets were used.

How might troglitazone act on islet hormone secretion and biosynthesis?

TZDs can influence gene transcription via PPARγ within 30 min of exposure (Burris et al. 1999). Although a functional peroxisomal proliferator response element (PPRE) has not yet been demonstrated in the PPI gene, PPARγ actions on insulin secretion and biosynthesis might be mediated by PPREs in the glucokinase as well as the GLUT2 gene (Kim et al. 2000, 2002). Both of these proteins are elements of the beta-cell glucose sensor and thus are directly involved in glucose-stimulated insulin secretion and biosynthesis (Newgard & McGarry 1995). Nonetheless, it has to be noted that troglitazone need not necessarily act through PPARγ (Bahr et al. 1996, Wang et al. 1999,
Brunnair et al. 2001, Davies et al. 2001). In that context, one must consider that we used the relatively high – albeit commonly employed in in vitro settings (Cnop et al. 2002, Kawai et al. 2002) – concentration of 10 µM troglitazone. As activation of PPARγ can be achieved with as little as 100 nM troglitazone (Adams et al. 1997), pharmacological effects of troglitazone which might surmount physiological effects of PPARγ activation cannot be fully excluded.

**Troglitazone effects under glucolipotoxic conditions**

Our results provide no evidence for positive direct effects of troglitazone on fatty acid-treated islets at high glucose levels in the short-term. A recent study by Patane et al. (2002) indirectly supports our findings with regard to insulin secretion. Here, FFA-treated islets showed elevated PPARγ levels, and insulin secretion was stimulated by PPARγ antagonists. In contrast to these studies and to our present findings, Higa et al. (1999) have demonstrated the capability of troglitazone – applied in vivo before onset of glucose intolerance as ‘prophylaxis’ or afterwards as ‘treatment’ – to restore the function of ZDF (fa/fa) rat islets with chronic fatty acid overload and defects in insulin secretion and content. Our in vitro model looking at direct short-term effects on previously healthy islets is not suitable for the analysis of such putative triglyceride-depleting effects of troglitazone.

**Conclusions**

Taken together, we have observed a decrease in insulin secretion and insulin biosynthesis after an acute treatment of isolated primary rat islets with troglitazone. This direct effect on the pancreatic islet was most marked at standard glucose levels. No clear beneficial effect of troglitazone was seen at glucolipotoxic conditions with simultaneously elevated glucose and oleate levels. We conclude from our results that troglitazone has a direct, not necessarily PPARγ-related, inhibiting effect on beta-cell insulin secretion and production predominantly at standard glucose levels. This ‘insulin-sparing’ effect might reduce the pathological basal hyperinsulinemia seen in insulin-resistant subjects and in early stages of adipogenic type 2 diabetes and thus, working hand in hand with the peripheral insulin-sensitizing effects, explain part of the clinical benefit of this TZD.

From our results, one would therefore expect the insulin-sparing effects of troglitazone to be most useful in prediabetic states. Appropriately, clinical studies examining the possibility of diabetes prevention by TZDs have already been initiated (Buchanan et al. 2000). The effects of troglitazone on glucagon secretion point to an elevation of alpha-cell endocrine activity at high glucose levels. The clinical significance of this remains unclear at present and warrants further investigation.

**Acknowledgements**

The authors acknowledge the excellent technical assistance of Iris Ottinger and Joachim Schweimer. The work was supported by grants from the Deutsche Forschungsgemeinschaft (DFG) (Bo 1379/2-1, Bo 1379/2-2).

**References**


Bollheimer LC, Skelly RH, Chester MW, McGarry JD & Rhodes CJ 1998 Chronic exposure to free fatty acid reduces pancreatic beta cell insulin content by increasing basal insulin secretion that is not compensated for by a corresponding increase in proinsulin biosynthesis translation. *Journal of Clinical Investigation* **101** 1094–1101.


Buchanan TA, Xiang AH, Peters RK, Kjos SL, Berkowitz K, Marroquin A, Goico J, Ochoa C & Azen SP 2000 Response of


Received in final form 4 March 2003
Accepted 17 March 2003