Lipophilicity as a determinant of thiazolidinedione action in vitro: findings from BLX-1002, a novel compound without affinity to PPARs

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Brunmair B, Staniek K, Lehner Z, Dey D, Bolten CW, Stadlbauer K, Luger A, Fürniss C. Lipophilicity as a determinant of thiazolidinedione action in vitro: findings from BLX-1002, a novel compound without affinity to PPARs. Am J Physiol Cell Physiol 300: C1386–C1392, 2011. First published February 23, 2011; doi:10.1152/ajpcell.00401.2010.—The pharmacology of thiazolidinediones (TZDs) seems to be driven not only by activation of peroxisome proliferator-activated receptor-γ (PPARγ), but also by PPARγ-independent effects on mitochondrial function and cellular fuel handling. This study portrayed such actions of the novel hydrophilic TZD compound BLX-1002 and compared them to those of conventional TZDs. Mitochondrial function and fuel handling were examined in disrupted rat muscle mitochondria, intact rat liver mitochondria, and specimens of rat skeletal muscle. BLX-1002 was superior to most other TZDs as an inhibitor of respiratory complex 1 in disrupted mitochondria, but had less effect than any other TZD on oxygen consumption by intact mitochondria and on fuel metabolism by intact tissue. The latter finding was obviously related to the hydrophilic properties of BLX-1002, because high potentials of individual TZDs to shift muscle fuel metabolism from the aerobic into the anaerobic pathway were associated with high ClogP values indicative of high lipophilicity and low hydrophilicity (e.g., % increase in lactate release induced by 10 μmol/l of respective compound: BLX-1002, ClogP 0.39, +10 ± 8%, not significant; pioglitazone, ClogP 3.53, +68 ± 12%, P < 0.001; rosiglitazone, ClogP 5.58, +137 ± 14%, P < 0.001). The observed specific properties of BLX-1002 could result from relatively strong direct affinity to an unknown mitochondrial target, but limited access to this target. Results suggest 1) that impairment of mitochondrial function and increased anaerobic fuel metabolism are unlikely to account for PPARγ-independent glucose lowering by BLX-1002, and 2) that higher lipophilicity of an individual TZD is associated with stronger acceleration of anaerobic glycolysis.

The glucose-lowering action of thiazolidinediones (TZDs) is attributed to their agonistic action on peroxisome proliferator-activated receptor-γ (PPARγ), a nuclear receptor that is expressed predominantly in adipose tissue and regulates adipogenesis. In muscle and liver, which are the quantitatively most important tissues for insulin-dependent glucose homeostasis, TZD-induced insulin sensitization seems to occur secondary to PPARγ-mediated changes in lipid handling and signal output from adipose tissue. While such fat-mediated mode of TZD action is undisputed, evidence accumulates that the pharmacology of TZDs could be driven not only by PPARγ activation, but also by PPARγ-independent and nongenomic effects on mitochondria (1–3, 5, 8, 14).

In vitro, TZDs have been shown to inhibit the activity of complex 1 of the respiratory chain and to accelerate anaerobic glycolysis in various cells and tissues, including skeletal muscle and liver (3, 8, 24, 25). This could at least in part relate to binding of TZDs to the mitochondrial protein mitoNEET, lack of which impairs the capacity of isolated mitochondria to utilize substrates of complex 1 (28).

Clinical relevance of such actions is discussed in the context of both, adverse and beneficial effects of TZDs. On the adverse side, a strong line of evidence points to mitochondrial dysfunction as the cause of the TZD-induced hepatotoxicity, which limits the clinical utility of some TZD compounds and has led to the removal of troglitazone from the market (14, 20). On the beneficial side, mitochondrial actions have been speculated to be potentially useful in the treatment of neurodegenerative diseases (11) and to trigger insulin sensitization. A connection between complex 1, mitochondrial function, and insulin sensitization is suggested by enhanced insulin sensitivity in genetically manipulated mice with deficient function of complex 1 and reduced tissue ATP levels (23), as well as by the insulin-sensitizing action of metformin, an inhibitor of complex 1 and mitochondrial oxygen consumption, which exhibits neither PPAR-agonist activity nor structural similarity with TZDs (3, 15, 17, 21).

Independently of the roles of specific mitochondrial targets like complex 1 or mitoNEET, it is puzzling that decreases in the cellular energy charge consistently precede insulin sensitization not only under exposure to drugs, but also in response to regular exercise, the hormone adiponectin, and pharmacological inducers or mimickers of cellular energy shortage (3, 22, 29, 31). Such consistent association supports what we refer to as the “energy charge hypothesis of insulin sensitization” (3), which suggests that reduced cellular energy availability can be a trigger for adaptations, which with some delay result in improved insulin sensitivity. The processes that connect energy shortage with insulin sensitization are not yet understood but seem to involve, e.g., activation of AMP-activated protein kinase (AMPK) and stimulation of mitochondrial biogenesis (27, 31).

From the above considerations, it is obvious that the exploration of direct effects on mitochondrial function and cellular fuel handling is an indispensable step in the pharmacological characterization of new TZD compounds. BLX-1002 is a novel...
tyrosine-coupled TZD that is neutral in charge, water-soluble (5.3 g/l), has a pKa value of 6.63, and does not structurally resemble any previously described TZD (detailed structure described in U.S. patent no. 6794401). Like other TZDs, BLX-1002 ameliorates hyperglycemia in rodent models of diabetes, but it neither shows relevant affinity to PPARγ nor does it induce weight gain as typically associated with PPARγ-mediated adipogenesis (6, 18, 19). The search for PPARγ-independent mechanisms of action has so far revealed that BLX-1002 potentiates glucose-stimulated insulin secretion from pancreatic islet cells (30). The present study explored some actions of BLX-1002 on mitochondrial function and muscle fuel metabolism along procedures allowing direct comparison to previously examined conventional TZDs. Comparison of different TZDs provided evidence that hydrophilicity/ lipophilicity is an important determinant of acceleration of anaerobic glycolysis in isolated skeletal muscle.

**MATERIALS AND METHODS**

**Rats.** Male Sprague-Dawley rats were purchased from the Core Unit for Biomedical Research, Division for Laboratory Animal Science and Genetics, Medical University of Vienna (Himberg, Austria). They were kept at an artificial 12 h-light/12 h-dark cycle at constant room temperature and, unless stated otherwise, provided with conventional laboratory diet and tap water ad libitum. All experiments were approved and performed according to local law and to principles of good laboratory animal care.

**Complex 1 activity in tissue homogenates.** Along procedures described in more detail earlier (3), frozen (−70°C) samples of the red part of gastrocnemius muscle from 6- to 8-wk-old rats were thawed, cut into small pieces, and homogenized (Polytron homogenizer, Kriens, Switzerland) in 0.1 mol/l K-phosphate buffer containing 0.3% wt/vol fatty acid-free bovine serum albumin (BSA; 30 mg tissue/ml; 0°C; pH adjusted to 7.4 with KOH). The homogenate was sonicated to disrupt cells and mitochondria (Labsonic U, B. Braun, Melsungen, Germany). An aliquot of 50 µl was supplemented with the indicated concentrations of BLX-1002 (provided by Bexel Pharmaceuticals, Union City, CA) and admixed to 1.8 ml K-phosphate buffer supplemented with 4 µl KCN (0.5 mol/l in water) and 4 µl NaN3 (1 mol/l in water). After equilibration (10 min; 30°C), 40 µl NADH (15 mmol/l in water; Fluka, Buchs, Switzerland) and 80 µl ubiquinone-1 (2.5 mmol/l in ethanol; Sigma, St. Louis, MO) were admixed to the tissue homogenate in a quartz cuvette, and the subsequent decrease in NADH was spectrophotometrically determined over 2 min. In this assay, NADH conversion is based on the reaction NADH + H+ + ubiquinone-1 → NAD+ + dihydroubiquinone-1, which is catalyzed by complex 1 and, hence, can be blocked by 1 µmol/l of the highly specific complex 1-inhibitor rotenone (reduction of NADH conversion by ~96 ± 2%).

**Oxygen consumption by isolated mitochondria.** Following a protocol that we have used previously (3), 3-mo-old rats were killed (cervical dislocation followed by decapitation) and livers were quickly excised for the isolation of mitochondria by tissue homogenization and differential centrifugation in isolation buffer (0.25 mol/l sucrose, 20 mmol/l triethanolamine, 1 mmol/l EDTA; pH 7.4; 4°C). The final suspension (30–40 g of mitochondrial protein/l) was kept at 4°C, and protein content (30 g) was determined by the Biuret method with the Biret method with BSA as standard. For the isolation of intact mitochondria, liver was used rather than skeletal muscle, because hepatic mitochondria can be obtained in much higher yields and behave qualitatively identical with regard to the bioenergetic functions examined here. Furthermore, TZD effects on mitochondrial function and lactate release have been shown to be similar in skeletal muscle and liver (3, 24, 25).

Oxygen consumption was measured with a Clark-type oxygen electrode. Mitochondria (1 g protein/l) were preincubated in isolation buffer additionally containing 0.3% wt/vol BSA and the indicated concentrations of BLX-1002 or rosiglitazone (3 min; 25°C). Buffers used to examine rosiglitazone and respective controls additionally contained 0.2% vol/vol dimethylsulfoxide (DMSO). To stimulate mitochondrial respiration, 4 mmol/l inorganic phosphate was added together with 5 mmol/l glutamate + 5 mmol/l malate (substrates for respiratory complex 1) or, alternatively, with 10 mmol/l succinate (substrate for complex 2) + 5 µmol/l rotenone (blocker of complex 1). After 3 min, mitochondrial respiration was accelerated by the addition of 200 µmol/l ADP, which allowed for oxidative phosphorylation and measurement of oxygen consumption in state 3 (i.e., with ATP synthesis). After quantitative consumption of added ADP, oxygen consumption was recorded in state 4 (i.e., without ATP synthesis). The energy-conserving capacity of mitochondria was determined by the respiratory control index (state 3/state 4). As a measure of the efficiency of mitochondrial ATP synthesis, the ratio of the total amount of ADP added per oxygen consumed during state 3 respiration was calculated. All measurements were done in duplicate and intra-assay control values were determined in parallel.

**Fuel metabolism of isolated muscle strips.** Fuel metabolism of native skeletal muscle was examined along procedures established at our laboratory (2, 3, 26). Food, but not water, was withdrawn from 6- to 8-wk-old rats for 3–4 h, before they were briefly anesthetized and killed by cervical dislocation for the preparation of the two longitudinal strips of soleus muscle per leg. Muscle specimens were immediately weighed (~25 mg/strip), tied under tension, and put into coated Erlenmeyer flasks provided with Cell Culture Medium 199 (pH 7.35, 5.5 mmol/l glucose; catalog no. M-4530, Sigma). The medium was additionally supplemented with 0.3% wt/vol BSA, 5 mmol/l HEPES, 300 µmol/l palmitate, 0.25% vol/vol ethanol (used to dissolve palmitate), 25,000 U/l penicillin G, 25 mg/l streptomycin, and 0.2 mg/l ciproflloxacin. Flasks were placed into a shaking water bath (37°C; 130 cycles/min) for pretreatment periods of 0.5 h (1 strip in 3 ml medium per flask) or 24 h (3 strips from different rats in 15 ml per flask). The pretreatment period was in the absence of insulin and, where indicated, in the presence of BLX-1002.

Immediately after the pretreatment period, muscles were transferred into identical medium for another hour (1 strip in 3 ml per flask). This measurement period was, if not stated otherwise, under stimulation with a maximally effective concentration of human insulin (Actrapid, Novo, Bagsvaerd, Denmark; 25 mmol/l after a 0.5 h pretreatment, 100 mmol/l after 24 h pretreatment), and in the presence of trace amounts of, alternatively, 0.2 µCi/ml d-[U-14C]glucose, 0.2 µCi/ml d-[U-14C]palmitic acid, or 0.4 µCi/ml 2-deoxy-d-[2,6-3H]glucose plus 0.06 µCi/ml d-[U-14C]sucrose (all from Amersham, Amersham, UK). After the measurement period, muscles were quickly removed from the flasks, blotted, and frozen in liquid nitrogen. Throughout the whole experiment, an atmosphere of 95% O2-5% CO2 was provided within the flasks.

An additional experiment was designed for direct comparison of BLX-1002 to other TZDs at a concentration of 10 µmol/l (troglitazone, pioglitazone, rosiglitazone, ciglitazone, darglitazone, and PNU-91325; all provided by Pfizer Research, St. Louis, MO). All conditions were as described above with a pretreatment period of 24 h, except that 0.1% vol/vol DMSO (required to dissolve lipophilic TZDs) was present in the media (also added to controls and to the water-soluble TZD BLX-1002).

Analytical methods have been outlined in more detail elsewhere (26). In short, rates of CO2 production from glucose or palmitate were measured as described above in separate runs as calculated from the conversion of [14C]glucose or of [14C]palmitate into 14CO2, which was trapped with a solution containing methanol and phenylenediamine (1:1). For the measurement of glycogen storage, muscle strips also used for the determination of glucose oxidation were frozen and lysed in 1 mol/l KOH at 70°C and
the net rate of glucose incorporation into glycogen (referred to as glycogen synthesis) was calculated from the conversion of \(^{14}C\)glucose into \(^{14}C\)glycogen. Intracellular accumulation of 2-deoxy-\(d\)-2,6-\(\beta\)glucose (referred to as glucose transport) was determined in another parallel run using \(d\)-[\(U\)-\(^{14}C\)]sucrose as a marker of extracellular space. Glycogen content prevailing at the end of the experiment was determined by degrading glycogen in the muscle lysate to glucose units with amylglucosidase, followed by measurement of glucose with an enzymatic kit (Human, Taunusstein, Germany). Rates of lactate release were calculated from lactate accumulated in the incubation medium as measured with the spectrophotometric lactate dehydrogenase method (7). For the determination of the energy charge, muscle strips were extracted with 3 mol/l perchloric acid, and ATP and phosphocreatine (PCr) were measured spectrophotometrically after neutralization with KOH (16).

Statistics. According to the exploratory character of the study, exploratory data analysis was applied and statistics were used in a descriptive sense. Results are given as means ± SE, and \(P\) values were calculated with two-tailed Student’s \(t\)-test (paired or unpaired, as appropriate) or with Pearson’s correlation. Where using the paired approach for the calculation of \(P\) values, figures depict mean intraindividual increases or decreases caused by the respective drug. The absolute rates obtained under control conditions are given in an electronic supplement (Supplemental Material for this article is available online at the Journal website). A \(P < 0.05\) was considered as significant.

RESULTS

Effects of BLX-1002 on complex 1 activity in tissue homogenates. In homogenates of rat skeletal muscle, BLX-1002 acutely suppressed the rotenone-sensitive conversion of NADH, which indicates impairment of the activity of complex 1 of the respiratory chain. Significant inhibition of complex 1 was induced by 10 \(\mu\)mol/l BLX-1002, and the amplitude of the effect increased dose dependently at higher concentrations, reaching a decrease to 63% of the control value at 100 \(\mu\)mol/l BLX-1002 (Fig. 1).

Effects of BLX-1002 on respiratory function of isolated mitochondria. In a suspension of mitochondria from rat liver, which contained substrates that enter the respiratory chain via complex 1 (glutamate and malate, via NADH), 100 \(\mu\)mol/l BLX-1002 caused a modest but significant decrease of oxygen consumption in state 3 (to 94% of the rate obtained under control conditions) (Fig. 2). In parallel, the energy-conserving capacity (respiratory control index) was significantly reduced to 92% of control. Concentrations of BLX-1002 required to trigger these effects were thus higher than those found to inhibit complex 1 activity in muscle homogenates (compare Figs. 1 and 2). With succinate that enters the respiratory chain via complex 2 (i.e., bypassing complex 1), no such effects of BLX-1002 were observed, suggesting a reduction of substrate flux through complex 1 but not complex 2.

In line with our previous report (3), 100 \(\mu\)mol/l rosiglitazone caused a distinct reduction of oxygen consumption in state 3, which was more pronounced in the presence of substrates for complex 1 than substrate for complex 2 (inhibition by \(-23.6 ± 1.4\%\) vs. \(-11.5 ± 3.7\%; \(P < 0.02\)). The energy-conserving capacity (respiratory control index) of isolated mitochondria was impaired by rosiglitazone to a similar extent under both conditions \([-14.8 ± 1.5\%\) vs. \(-12.5 ± 1.2\%; not significant (NS)]

Effects of BLX-1002 on energy charge of isolated rat muscle. Possibly because of the above described impairment of mitochondrial function, 24 h of exposure to 100 \(\mu\)mol/l BLX-1002 reduced the cellular energy charge of rat skeletal muscle in vitro as indicated by a significant decrease of the PCr/ATP ratio to 78% of the control value (\(P < 0.05\); Fig. 3). Under the same experimental conditions, more than 10-fold lower concentrations of rosiglitazone, which are known to affect fuel metabolism in this experimental setting (Refs. 2 and 3 and this study), triggered significant decreases in the cellular energy state.

Effects of BLX-1002 on fuel metabolism of isolated rat muscle. When isolated specimens of rat skeletal muscle were exposed to BLX-1002 for 24 h, the observed changes in fuel metabolism were as typically occurring in response to impaired cell respiration and loss of cellular energy charge (Figs. 4 and 5). At 100 \(\mu\)mol/l BLX-1002, this included reductions by almost 25% in glucose and fatty acid oxidation to \(\text{CO}_2\) along with a compensatory ~50% increase in anaerobic glycolysis (lactate release). Higher carbohydrate requirements for anaerobic ATP generation were reflected by stimulation of glucose transport (increased by 69%) and glycogen depletion (rate of glycogen synthesis as well as glycogen content reduced by ~25%). The response was rather modest at a lower concentration of 30 \(\mu\)mol/l BLX-1002 (Fig. 4). In agreement with previous findings about other TZDs tested along the same protocol (2, 3, 9), similar but more modest effects occurred within 1.5 h (Fig. 5, top graphs). Also in line with our previous studies, the effect persisted without concomitant insulin stimulation and can therefore not be interpreted as a change in insulin sensitivity (Fig. 5, bottom graphs).

Interdependence between ClogP values and effects on fuel handling. The effects of different TZD compounds (10 \(\mu\)mol/l) on fuel metabolism of isolated rat muscle were examined in a comparative manner, allowing the analysis of interdependence between their lipophilic/hydrophilic properties as indicated by the ClogP value and their efficacies to affect aerobic and anaerobic fuel metabolism (Fig. 6). ClogP values were specified by the companies providing the compounds. Significant associations were observed between ClogP values of the individual TZD compounds and their corresponding inhibitory action on palmitate oxidation \((r = -0.815, P < 0.03)\), as well as between ClogP values and stimulatory action on lactate release \((r = 0.813, P < 0.03); \text{Fig. 6})

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Fig. 1. BLX-1002 inhibits mitochondrial complex 1. Effects of BLX-1002 on the enzymatic activity of respiratory complex 1 in homogenates of the red part of rat gastrocnemius muscle, which were sonicated to disrupt cell and mitochondrial membranes. Data are means ± SE of differences vs. intraindividual control; \(n = 10\) each; *\(P < 0.05\), †\(P < 0.001\) vs. control (\(= 0\)).
DISCUSSION

Our results show that BLX-1002 1 inhibits complex 1 activity in tissue homogenate, 2) affects respiratory function in isolated mitochondria in a manner suggesting reduced NADH conversion by complex 1, and 3) decreases the cellular energy charge and shifts fuel metabolism from aerobic towards anaerobic pathways in isolated rat muscle. From a mechanistic point of view, all these attributes resemble the effects on mitochondrial function and muscle fuel metabolism previously documented for troglitazone and other conventional TZDs (2, 3, 8, 25).

Although we have not directly measured cell respiration in muscle specimens exposed to BLX-1002, reduced cellular energy charge along with a shift of fuel metabolism from mitochondrial oxidation toward lactate production resembles the typical responses to a respiration inhibitor or hypoxia. This includes accelerated glycogen depletion and glucose uptake, which are established adaptive responses to energy shortage (2, 10, 13). We found these effects of BLX-1002 on muscle metabolism to be independent of concomitant insulin stimulation and to be more pronounced after prolonged expo-
sure. All these characteristics of the responses to the hydrophilic TZD BLX-1002 resemble the at least partly non-genomic effects previously ascribed to the highly lipophilic TZD troglitazone (2).

While the pattern of response observed for BLX-1002 thus seems common for the whole class of TZDs, comparison of relative efficacies of the individual compounds reveals interesting observations. With respect to inhibition of respiratory complex 1 in tissue homogenate, BLX-1002 clearly outmatched most other TZDs. Among eight TZDs that we have tested so far along the same protocol, only rosiglitazone and BLX-1002 significantly inhibited complex 1 activity at concentrations of 10 and 30 μmol/l (Ref. 3 and this study).

Accordingly, established TZDs except rosiglitazone were clearly less effective than BLX-1002 at 100 μmol/l (comparison to results from Ref. 3: BLX-1002, −38 ± 7%; vs. troglitazone, −21 ± 3%; P < 0.001; vs. pioglitazone, −12 ± 4%; P < 0.001; and vs. rosiglitazone, −54 ± 7%, P = 0.06, NS).

Superiority of rosiglitazone over BLX-1002 persisted also in the other experimental settings, but apart from this, very different rankings were obtained with intact specimens of skeletal muscle exposed to various TZDs for 24 h. In isolated muscle, the water-soluble compound BLX-1002 had the weakest effect on fuel handling, whereas the very lipophilic compound troglitazone was the strongest of all TZDs examined so far (Refs. 2 and 3 and this study). Accordingly, our analysis revealed an association between the ClogP value of a given

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**Fig. 4.** BLX-1002 enhances anaerobic fuel metabolism. Insulin-stimulated rates of glucose transport, lactate release, glucose and palmitate oxidation, and glycogen synthesis in rat soleus muscle after 24 h exposure to BLX-1002; glycogen content was measured at the end of the experiment. Data are means ± SE of differences vs. intraindividual control; n = 6–30 each; *P < 0.05, †P < 0.01, ‡P < 0.001 vs. control (= 0).

**Fig. 5.** BLX-1002 affects fuel handling rapidly and independently of insulin stimulation. Insulin-stimulated (top graphs) and basal (bottom graphs) rates of lactate release, glucose oxidation, and glycogen synthesis in rat soleus muscle after 1.5 h exposure to BLX-1002. Data are means ± SE of differences vs. intraindividual control; n = 6 each; *P < 0.05, †P < 0.01 vs. control (= 0).
TZD and its influence on palmitate oxidation and lactate production by isolated muscle specimens. In contrast to what was seen in intact muscle, the very hydrophilic BLX-1002 as well as the very lipophilic troglitazone were mediocre inhibitors of complex 1 in muscle homogenates, which argues against ClogP dependence of direct interaction with mitochondrial particles.

One possible explanation for such divergence is that TZD efficacy in sonicated homogenates, which contain disrupted mitochondria and are exposed to the drug briefly, relates to the potential of the individual compound to directly interact with a molecular target and to trigger early events within the mitochondrial membrane. On the other hand, dependence of efficacy on the hydrophilic/lipophilic properties of the respective molecule, as it was observed in intact muscle subjected to prolonged exposure, hints at an influence of access to and/or accumulation at the relevant intracellular compartment. Although this interpretation implicates that mitochondrial TZD actions are not determined by ClogP alone, it would explain why the high potential of the hydrophilic BLX-1002 to affect mitochondrial particles does not translate into a similar effectiveness in intact tissue and intact mitochondria. Furthermore, our study does not exclude that mechanisms other than complex 1 inhibition contribute to TZD-induced acceleration of anaerobic glycolysis. For example, there is evidence that pioglitazone can impair mitochondrial oxygen consumption via a target upstream of complex 1 (8). While the precise molecular mechanisms responsible for the mitochondrial effects of TZDs thus still await their full elucidation, our results clearly show that even very high concentrations of BLX-1002 have only modest effects on intact mitochondria and cells. This obviously argues against a major role of any mitochondrial effect in the glucose-lowering activity of BLX-1002 in vivo.

Troglitazone, which in line with its highly lipophilic properties has stronger effects on fuel handling than other TZDs, was prescribed to patients at relatively high doses. Hence, our results and interpretations are compatible with the idea that the mechanisms responsible for effects on fuel metabolism in our isolated muscle-protocol could also account for the dangerous hepatotoxic effects of this drug (14). Accordingly, preclinical studies with the hydrophilic compound BLX-1002 did not bring forward any issues of hepatotoxicity, including that changes in circulating liver enzymes were not observed (19). All this suggests that the combined consideration of the required therapeutic dose, the ClogP value, and the effects on mitochondrial function in vitro could at a very early stage be predictive of the hepatotoxic potential and, hence, the clinical utility of a novel TZD.

But the evidence that mitochondrial TZD actions and increased anaerobic glycolysis could be responsible for unwanted side effects of TZDs does not exclude that they could also contribute to beneficial actions. Indeed, therapeutic usefulness of mitochondrial TZD actions has been hypothesized in the context of metabolic and neurodegenerative diseases, which stimulated efforts to design and develop TZDs based on their interaction with mitochondria rather than PPARγ (4, 11). BLX-1002 seems to be a PPARγ-sparing TZD, but its very weak effects on mitochondrial function and fuel metabolism in vitro argue against a relevant contribution of such actions to antihyperglycemic activity in experimental animals. Our results are therefore compatible with the idea that the pharmacology of BLX-1002 is sufficiently explained neither by PPARγ activation nor by mitochondrial actions, but must rely on another mechanism as could be the amplification of glucose-stimulated insulin secretion from pancreatic β-cells described in vitro (30).

In conclusion, the lipophilicity/hydrophilicity of an individual TZD compound seems to be an important determinant of its potential to shift fuel metabolism from aerobic to anaerobic pathways. Consequently, modulation of mitochondrial function is unlikely to make a relevant contribution to the presumably PPARγ-independent glucose lowering effects of the hydrophilic TZD BLX-1002.

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DISCLOSURES

D. Dey was employee of Bexel and had an employee stock option plan during his full tenure. C. W. Bolton was previously employed by and currently holds stock in Pfizer, Inc. He is currently employed by BioGenerator, which holds stock in pharmaceutical companies. A. Lugner has received honoraria from Pfizer as consultant and for speaking at scientific meetings. C. Führnsinn has received an honorarium for a lecture at Pfizer in 2006.

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