Glitazones Differentially Regulate Primary Astrocyte and Glioma Cell Survival

IN INVOLVEMENT OF REACTIVE OXYGEN SPECIES AND PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-γ [S]

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The glitazones or thiazolidinediones are ligands of the peroxisome proliferator-activated receptor γ (PPARγ). The glitazones are used in the treatment of diabetes, regulate adipogenesis, inflammation, cell proliferation, and induce apoptosis in several cancer cell types. High grade astrocytomas are rapidly growing tumors derived from astrocytes, for which new treatments are needed.

We determined the effects of two glitazones, ciglitazone and the therapeutic rosiglitazone, on the survival of serum-deprived primary rat astrocytes and glioma cell lines C6 and U251, which were assessed by the methyl-thiazolyl tetrazolium assay and lactate dehydrogenase release. Rosiglitazone (5–20 μM) decreased survival of glioma cells without affecting primary astrocytes, whereas ciglitazone at 20 μM was toxic for both cell types. Ciglitazone at 10 μM was cytoprotective for primary astrocytes but toxic to glioma cells. Cell death induced by ciglitazone, but not rosiglitazone, presented apoptotic features (Hoechst staining and externalization of phosphatidylserine). Two mechanisms to explain cytotoxicity were investigated: activation of PPARγ and production of reactive oxygen species (ROS). PPARγ does not seem to be the main mechanism involved, because the order of efficacy for cytotoxicity, ciglitazone > rosiglitazone, was inverse of their reported affinities for activating PPARγ. In addition, GW9662, an inhibitor of PPARγ, only slightly attenuated cytotoxicity. However, the rapid increase in ROS production and the marked reduction of cell death with the antioxidants ebseilen and N-acetylcysteine, indicate that ROS have a key role in glioma cytotoxicity. Ciglitazone caused a dose-dependent and rapid loss (in minutes) of mitochondrial membrane potential in glioma cells. Therefore, mitochondria are a likely source of ROS and early targets of glitazone cytotoxicity. Our results highlight the potential of rosiglitazone and related compounds for the treatment of astroglia.

The glitazones or thiazolidinediones are a family of synthetic compounds that include ciglitazone, troglitazone, pioglitazone, and rosiglitazone. The two latter drugs are currently used for the treatment of type 2 diabetes mellitus because of their effectiveness in controlling hyperglycemia (1). On top of their antidiabetic actions, glitazones show potent biological effects as anti-inflammatory drugs (2) and as regulators of cell survival. Depending on the cell type, they can promote either cytoprotection or cytotoxicity. Thus, whereas ciglitazone and rosiglitazone protect B and T lymphocytes from apoptosis after growth factor withdrawal (3), rosiglitazone increases apoptosis in human monocyte-derived macrophages (4). Glitazones induce growth arrest and apoptosis in a broad spectrum of tumor cells (5–7).

Regarding their mechanisms of action, glitazones are potent ligands of the peroxisome proliferator-activated receptor γ (PPARγ), which belongs to the nuclear receptor family together with the other two known PPAR isoforms, α and β (also called δ). Although PPARγ was originally known for its role in adipocyte differentiation and lipid metabolism, it was later found to be more widely expressed and to play functions related to cell death, differentiation, energy homeostasis, and inflammatory response (1, 8–12). Endogenous, natural ligands of PPARγ include the 15-deoxy-Δ12,14-prostaglandin J2 (15d-PG J2) as well as several polyunsaturated fatty acids (11, 13). However, a growing number of reports have recently argued against the implication of PPARγ in several effects of glitazones and 15d-PG J2 (14–19).

The brain expresses all PPAR isoforms; of relevance to brain disease is that PPARγ ligands down-regulate the expression of proinflammatory molecules and ameliorate neuronal death in animal models of neurodegenerative diseases (20). Furthermore, a clinical trial underway examines the potential of rosiglitazone to improve neuronal survival in Alzheimer’s disease. The roles of astrocytes and brain in order to assess the potential functions other than nurturing neurons are being found. Thus, they are believed to be relevant in neurological diseases with an immunological component (21, 22). Ciglitazone, one of the

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earlier compounds in the glitazone family, has been found to reduce cellular viability of primary astrocytes (23). However, the effects of rosiglitazone on astrocyte viability have not been tested yet. The main goal of this study was to compare the effects of rosiglitazone and ciglitazone on the survival of astrocytes and astroglialoma cells and to identify the mechanisms behind their toxicity. Our results indicate that rosiglitazone is less toxic than ciglitazone, and that glioma cells in culture are more sensitive than primary astrocytes. Although the most established mechanism of action of glitazones is activation of PPARy, we found that the major mechanism mediating glitazone-induced glial cell death is the generation of reactive oxygen species (ROS). Because glitazones cause mitochondrial depolarization in our system, mitochondria are the most likely source of these ROS. Finally, the toxicity of glitazones on glioma cells and the relative resistance of astrocytes suggest that glitazones may hold therapeutic potential for the treatment of astroglomas, which are among the most common brain tumors.

EXPERIMENTAL PROCEDURES

Chemicals—Rosiglitazone was a generous gift of GlaxoSmithKline. 15d-PG J2, ciglitazone, and WY14643 were purchased from Tocris (Ballwin, MO). GW9662 was obtained from Cayman Chemical (Ann Arbor, MI). Dichlorodihydrofluorescein diacetate (H2DCF-DA), tetramethylrhodamine ethyl ester (TMRE), and annexin V conjugated with biotin (Leica DMRXA), with excitation centered at 360 nm, a 400-nm beam splitter, and emission longer than 425 nm.

Flow Cytometry Analysis of Apoptotic Cells—C6 glioma cells and primary astrocytes were harvested and stained simultaneously with annexin V-FITC (Promega). Annexin V-stained cell populations were detected by annexin V conjugates. Cells that were positive for annexin V and negative for PI were considered to be early apoptotic. Annexin V-negative/PI-negative cells were considered living cells, and cells positive for both annexin V and PI were designated dead cells (neurotic plus late apoptotic cells).

Western Blotting—Cell lysates were loaded onto a 10% SDS-polyacrylamide gel (20 μg/lane), electrophoresed, and subsequently transferred to nitrocellulose membranes. Cells were stained with pCMV-β-galactosidase to normalize the variations in cell number and transfection efficiency. Rosiglitazone, ciglitazone, or vehicle were added 16 h after transfection. Cells were lysed 24 h after treatments, and the luciferase and β-galactosidase assays were performed according to the vendor instructions (Promega). In all experiments, cells were plated 24 h before treatments, and serum was removed from cell media at the time of drug additions. For the methyl-thiazolyl tetrazolium (MTT) and the lactate dehydrogenase (LDH) assays cells were plated into 96-well culture plates. Six replicate wells were used for each treatment. Cell density was 105 cells/well for glial cell lines and 1.5 × 106 cells/well for primary astrocytes. Both assays were performed 48 h after treatment. For Western blot analysis, cells were lysed 36 h after seeding cells and the relative resistance of astrocytes suggest that glitazones may hold therapeutic potential for the treatment of astrogloma, which are among the most common brain tumors. The nuclear morphology of cells was studied by using the cell-permeable DNA dye Hoechst 33342. Cells with homogeneously stained nuclei were considered to be viable, whereas the presence of chromatin condensation and/or fragmentation was indicative of apoptosis. Hoechst 33342 staining as well as contrast images were obtained by using a fluorescence photomicroscope.
fluorescence inverted microscope (DMIRE-2, Leica Microsystems, Germany) equipped with an oil immersion 40× objective (numerical aperture 1.25). Fluorescence was excited at 545 nm using a monochromator (Hamamatsu Photonics, Japan), and emitted light was collected by a charge-coupled device camera (Orca-ER, Hamamatsu Photonics, Japan). The interference filter used for emission was 635 nm, and the dichroic mirror was 570DRL (Omega Optical). Regions of interest (over the nuclei and perinuclear cytoplasm) were selected manually, and pixel intensities were spatially averaged after background subtraction. A binning of two was used to improve signal/noise and minimize photobleaching of TMRE and photodamage to the cells. All images and data were acquired and analyzed by using the Aquacosmos software (Hamamatsu Photonics, Japan).

**Statistical Analysis**—Results are expressed as percentage of control treated with the appropriate vehicle (Me2SO). Values represent mean ± standard deviation (S.D.) of 3–6 replicate wells. Data were analyzed by one-way analysis of variance followed by a Student’s *t* test when multiple comparisons were made.

**RESULTS**

Effects of Glitazones on the Viability of Astroglial Cells—Cell viability was assessed by the MTT reduction method 48 h after glitazone treatment of serum-deprived rat primary astrocytes, rat glioma C6 cells, and human glioma U251 cells. Serum withdrawal progressively reduces the viability of cells, allowing the study of both the cytotoxic and cytoprotective actions of glitazones. Our results showed that ciglitazone and rosiglitazone affect astroglial cell viability in a dose-dependent and cell-specific manner (Fig. 1, A and B). Thus, ciglitazone caused death of glioma cells at doses (5–15 μM) that did not decrease astrocyte survival. However, at 20 μM, ciglitazone was toxic to both glioma cells and primary astrocytes. Rosiglitazone had no effect on primary astrocyte viability but was toxic for both glioma cell lines, with the highest effect at the dose of 20 μM. In any case, rosiglitazone was much less cytotoxic than ciglitazone. Ciglitazone is, therefore, a potent inhibitor of astroglial survival, whereas rosiglitazone is a weaker inhibitor and affects only glioma cells. An interesting observation was that not all concentrations of ciglitazone had a toxic effect on primary astrocytes. At 10 μM ciglitazone, MTT values were higher than in vehicle-treated controls. This increase in MTT reduction was not due to a stimulation of astrocyte proliferation, because ciglitazone decreased thymidine incorporation in these cells. The cytoprotective effect of ciglitazone on primary astrocytes was never observed with rosiglitazone. We determined the cell viability of C6 cells and astrocytes treated with other PPAR ligands (see Fig. 1, C and D): the PPARγ agonist 15d-PG J2 and

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the PPARα agonist WY14643. The ligand 15d-PG J₂ was able to induce cell death in a dose-dependent manner in all glial cell types tested, being more toxic for primary astrocytes than for C6 glioma cells. In contrast, cell viability was unaffected by WY14643 at all doses and cell types studied (up to 100 μM). Cell protection was never observed after treatment with 15d-PG J₂ or WY14643. These results indicate that PPARγ ligands other than glitazones, but not PPARα ligands, can cause astroglial cell death in our experimental system. However, the decrease in cell survival induced by ciglitazone was more severe than that caused by either 15d-PG J₂ or rosiglitazone, and the cell type selectivity of ciglitazone was different from that of 15d-PG J₂, which suggested that different mechanisms were at work.

MTT reduction depends not only on cell death, but also on variations of cell number which may occur in cycling populations and on the function of mitochondrial dehydrogenases. Therefore, we measured LDH released from glial cells to the medium as another marker of cell viability, though less affected by proliferative and metabolic changes. Although ciglitazone at concentrations of 15 and 20 μM increased LDH leakage in C6 cells more than 70%, primary astrocytes were affected only with 20 μM ciglitazone. Rosiglitazone at 10 and 20 μM increased LDH release in C6 cells more modestly than ciglitazone (less than 30% increase) and had no effect on primary astrocytes at any of the doses studied. Thus, the results of the LDH assays (Fig. 2) confirm the MTT data (Fig. 1), suggesting that ciglitazone strongly decreases glioma cell viability, that rosiglitazone is a weaker inhibitor, and that astrocytes are less sensitive to glitazone toxicity than glioma cells.

Induction of Apoptotic Cell Death by Glitazones—Cell death caused by glitazones was monitored by Hoechst 33342 staining (Fig. 3). Glioma cells and primary astrocytes treated for 48 h with 20 μM ciglitazone exhibited apoptosis features consisting of chromatin margination and condensation in cell nuclei. However, we were unable to detect glioma cells or primary astrocytes showing condensed nuclear material or other signs of increased apoptosis after rosiglitazone treatment (20 μM). Both glitazones decreased cell density in C6 glioma cells, as observed in interference contrast images. In addition, ciglitazone decreased cell density in primary astrocytes and induced cell shrinkage in both astrocytes and C6 glioma cells.

To further characterize apoptosis, we performed an annexin V/PI assay by flow cytometry (Fig. 4A), which allows quantitative and statistical analysis of live, apoptotic, and dead cell populations (Fig. 4B). Ciglitazone at 20 μM induced apoptosis in both C6 glioma and primary astrocyte cells. After 24 h of ciglitazone exposure, the percentage of early apoptotic and dead glioma cells was, respectively, 150 and 660% higher than controls. When the treatment with ciglitazone was extended to 48 h, we observed a reduction in the number of early apoptotic cells and a further increase in the number of dead cells (results not shown), which can be explained by a progression of cells to a later apoptotic stage with time. Primary astrocytes were also sensitive to 20 μM ciglitazone, although their response was delayed compared with glioma cells. After 48-hour treatment, there was an 85% increase in early apoptotic cells and a 180% increase in dead astrocytes. Rosiglitazone (20 μM) caused an increase in the number of dead glioma cells close to 900% within 24 h. However, glioma cells did not show any statistically significant increase in the number of early apoptotic cells 24 or 48 h after rosiglitazone addition (results only shown for 24-hour treatments). In conclusion, rosiglitazone is able to cause death of glioma cells but, unlike ciglitazone, we did not find any evidence of apoptosis. Also, in agreement with the Hoechst staining data (Fig. 3), rosiglitazone did not significantly change the percentage of apoptotic or dead cells in primary astrocytes.

Dependence of Astroglial Cell Death Caused by Glitazones on PPARγ—To explore whether PPARγ mediates the actions of glitazones on astroglial cell viability described above, we first evaluated PPARγ expression by the astroglial cells used in our experiments. Constitutive expression of PPARγ in primary astrocytes and glioma cell lines C6 and U251 was examined by Western blotting (Fig. 5A). Our results confirm the presence of PPARγ in all cell types studied. PPARγ appeared as a single band of 50 kDa, and its identity was confirmed by comparison with PPARγ induced in primary macrophage cultures stimulated with lipopolysaccharide and interferon γ (data not shown). These results are in agreement with previous reports, which have detected PPARγ in astroglial cells by PCR (23, 31) and Western analysis (23, 32). Moreover, PPARγ expressed in glioma C6 cells was transcriptionally functional, as demonstrated by ligand-dependent induction of a luciferase reporter gene under the control of a thymidine kinase promoter containing a PPARγ-response element (Fig. 5B).

The fact that ciglitazone strongly decreases glioma cell viability, whereas rosiglitazone is a weaker inhibitor, is in sharp contrast with their potency as agonists of PPARγ, because rosiglitazone is about 70-fold more potent than ciglitazone (EC₅₀ are 43 nM and 3 μM, respectively) (33). This suggests that PPARγ expressed in astroglial cells is not the major mechanism causing glitazone cytotoxicity. We further addressed this point by analyzing the effects of GW9662 on ciglitazone-induced cytotoxicity. GW9662 is an inhibitor of PPARγ with high affinity and selectivity and can fully abrogate PPARγ-dependent effects (34). GW9662 (5 μM) increased the survival of glioma cells and primary astrocytes treated with ciglitazone (20 μM) by...
32 and 28%, respectively, as evaluated by the MTT assay (Fig. 6). Because this represents only a partial blockage of cytotoxicity, these results imply that PPARγ activation has a limited participation in such ciglitzone actions, and that alternative PPARγ-independent mechanisms mediate glitazone-induced astroglial cell death.

**Glitazones Induce ROS in Astrocytes and Glioma Cells**—ROS generation is a common cellular mechanism for multiple death pathways (35, 36). To investigate whether ROS generation is involved in the effects of glitazones on astroglial viability, ROS production was evaluated in H2DCF-loaded C6 glioma cells and primary astrocytes by flow cytometry after incubation for 1 h with 20 μM ciglitazone, 20 μM rosiglitazone, or vehicle (0.2% Me2SO) (Fig. 7). Both ciglitazone and rosiglitazone induced an increase in intracellular ROS steady-state levels in C6 cells and primary astrocytes, but ciglitazone produced a larger ROS increment (210% for C6 cells and 140% for astrocytes) than rosiglitazone (77% for C6 cells and 61% for astrocytes). Therefore, a positive correlation was observed between the levels of ROS generated by each glitazone and their ability to cause apoptosis and astroglial cell death. Ciglitazone, which induces the highest ROS production, causes the highest toxicity in astroglial cells.

**ROS Generation by Glitazones Is Followed by a Decrease in GSH**—The normal reducing environment in the cytoplasm and mitochondria is maintained by the ubiquitous tripeptide thiol GSH, and ROS usually trigger GSH oxidation. We measured GSH in C6 cells to evaluate the redox status of astroglial cells treated with glitazones (Fig. 8). Exposure to 20 μM ciglitazone or rosiglitazone for 9 h significantly decreased the levels of GSH in cells. Ciglitazone decreased GSH (by 60%) more than rosiglitazone (32%), which correlates with the amount of ROS produced by each glitazone. The effects of glitazones on GSH were delayed with respect to ROS induction, because reduced GSH was only slightly decreased (not significantly) after 1 h of treatment with either glitazone, suggesting that continuous ROS production finally leads to GSH depletion. Therefore, our data from both GSH and ROS measurements show that glitazones are able to alter the redox status of astroglial cells within hours.

**ROS Activation Is Involved in the Cytotoxicity of Glitazones**—To test whether ROS were involved in the reduction of cell viability observed after glitazone exposure, we used the antioxidants N-acetylcysteine and ebselen. The former provides cysteine for GSH synthesis and spares GSH by reacting with ROS, whereas the latter has GSH peroxidase-like activity. Primary astrocytes were pretreated for 12 h with N-acetylcysteine (1 mM, Fig. 9A) or for 1 h with ebselen (5 μM, Fig. 9B) before the addition of 20 μM ciglitazone. Cell viability, assessed 48 h after ciglitazone stimulation by the MTT reduction assay, revealed that antioxidant treatment almost abolished cytotoxicity induced by ciglitazone in primary astrocytes (Fig. 9) and markedly reduced it in C6 cells (results not shown). These results implicate ROS as a major pathway in the cytotoxic response of astroglial cells to glitazones.

**Ciglitazone Causes a Loss of Mitochondrial Transmembrane Electric Potential Gradient (∆ψmt)**—Mitochondria have been recognized to be sources of H2O2 and superoxide radical (38) and are key players in the initiation of apoptosis in many systems. Therefore, we examined the effect of ciglitazone on ∆ψmt by imaging C6 cells loaded with the live-cell indicator TMRE. This indicator accumulates in mitochondria driven by ∆ψmt, and it can suffer self-quenching when its concentration within this organelle is high (39). Upon depolarization, TMRE exits mitochondria and a new equilibrium is reached between the intracellular (nucleus and cytoplasm) and the extracellular compartment, given the plasma membrane electric potential gradient. If there is self-quenching in mitochondria, fluoress-
cence will initially rise during depolarization; however, if there is no self-quenching, fluorescence will decrease during depolarization. Therefore, to interpret unambiguously the fluorescence changes, we used the mitochondrial uncoupler (protonophore) FCCP as a positive control for depolarization within each experiment. Control cells loaded with TMRE showed staining of mitochondria and very little fluorescence in the cytoplasm and nucleus (Fig. 10A). Treatment with FCCP resulted in a rapid loss of mitochondrial staining and increase in cytoplasmic/nuclear and extracellular fluorescence. Because C6 cells are very flat and extended on their substrate, the rise in extramitochondrial fluorescence was best observed over the cell nuclei. We used the ratio of fluorescence integrated over the nuclear region and the perinuclear region (which comprises both cytoplasm and mitochondria) as a sensitive measure of \( \Delta \psi_M \). Depolarization with FCCP results in about a seven-fold increase in this ratio (Fig. 10B). Ciglitazone caused a rapid (within minutes) and dose-dependent loss of \( \Delta \psi_M \) in C6 glioma cells, because the mitochondrial pattern turned into a diffuse cytoplasmic and nuclear staining (Fig. 10A), and the nuclear/perinuclear fluorescence ratio increased (Fig. 10B). It is well established that some inhibitors of the respiratory chain increase superoxide anion and \( \text{H}_2\text{O}_2 \) production by mitochondria and loss of \( \Delta \psi_M \) (40, 41). Because we found that ciglitazone treatment results in both increased levels of ROS and mitochondrial depolarization, it is mechanistically plausible that ciglitazone acts by inhibiting the respiratory chain.

**DISCUSSION**

In our model of astroglial cell death by serum deprivation, a general conclusion from our current findings is that astroglial cell lines are more sensitive to cytotoxicity induced by glitazones than primary astrocytes. Thus, ciglitazone induced cell damage in both glioma cell lines tested (rat, C6; and human, U251). However, ciglitazone had a dual effect on the survival of primary astrocytes, because it caused a pronounced decrease in viability at 20 \( \mu \text{M} \), whereas it increased survival at 10 \( \mu \text{M} \). This suggests that ciglitazone can activate multiple pathways involved in astrocyte survival and death. In agreement with our results, two previous studies have shown that ciglitazone causes apoptosis in human malignant astrocytoma cell line T98G (23) and astrocytoma C6 cells (32). There are reports showing cytoprotection (31) or apoptosis (23) in primary astrocytes, but these discrepancies may be due to the different species used and the presence or absence of serum during glitazone treatment, because glitazones bind avidly to plasma proteins.

The second general conclusion from our study is that rosiglitazone, which is a therapeutic drug, showed cytotoxicity in glioma cells, but, in contrast to ciglitazone, it did not affect...
Glitazones Regulate Astroglial Survival

The use of the low-affinity PPARγ inhibitor 2,2-bis(4-(2,3-epoxypropoxy)phenyl)propane had claimed PPARγ involvement in ciglitazone-induced cell death in both primary murine astrocytes and glioma cells (32). However, in our results, the order of efficacy for cytotoxicity was ciglitazone > rosiglitazone, which is inverse of their reported affinities for binding and activating PPARγ. Rosiglitazone is a potent PPARγ agonist (EC50 = 43 nM), and full PPARγ activation would be expected at concentrations at least one order of magnitude lower than those that cause astroglial toxicity in this study. The fact that rosiglitazone causes less cytotoxicity than ciglitazone, which has an EC50 of 3 μM, argues against PPARγ as a key player in glitazone-induced astroglial death. By the same reasoning, ciglitazone-induced apoptotic death seems independent of PPARγ activation because rosiglitazone did not cause any significant apoptosis. Moreover, pretreatment with the high affinity PPARγ antagonist GW9662 could only partially rescue cells from death caused by glitazones, thus favoring the hypothesis that astroglial death induced by glitazones is not dependent on PPARγ.

Several of our findings suggest that glitazones cause cytotoxicity in astroglial cells through ROS generation. (i) Our cytometric analysis using H2DCF-loaded cells indicates that both ciglitazone and rosiglitazone increase the steady-state ROS levels in glioma C6 cells and primary astrocytes as soon as one hour after drug addition. There is a positive correlation between the levels of ROS and the severity of the cytotoxicity caused by glitazones: ciglitazone was more effective than rosiglitazone to induce both ROS and cell death. (ii) Glitazones disrupt the redox status by depleting intracellular GSH as a consequence of the increase in ROS levels. (iii) The last piece of evidence indicating that ROS generation is involved in the loss of cell viability is that ciglitazone cytotoxicity was significantly prevented by the antioxidants ebselen and N-acetyl cysteine (Fig. 9).

Regarding the mechanism of ROS production, they can be of mitochondrial or extramitochondrial origin (i.e. NADPH oxidase). We investigated mitochondrial function because mito-
Chondria are a likely source of ROS and can be involved in the initiation of apoptosis in a m-dependent fashion. Ciglitazone caused a dose-dependent and rapid (within tens of seconds) loss of H\textsubscript{m} in C6 glioma cells. We have not identified the exact target of ciglitazone in producing this depolarization, but it is known that many mitochondrial inhibitors increase the steady-state levels of H\textsubscript{2}O\textsubscript{2}. For instance, antimycin A promotes the autooxidation of ubisemiquinone in the respiratory chain to generate superoxide anion, which is converted to H\textsubscript{2}O\textsubscript{2} by superoxide dismutases (40). This inhibition of the respiratory chain also results in mitochondrial depolarization. It is mechanistically plausible that ciglitazone inhibits mitochondria in such a way, because we found a rapid loss of \Delta\psi\textsubscript{m} and subsequently increased levels of ROS (Figs. 7 and 10, respectively).

In agreement with this proposed mechanism, troglitazone and pioglitazone were found to inhibit state III respiration in isolated brain mitochondria (31). Furthermore, a very recent study has identified a novel binding site of glitazones in mitochondria, using tritiated pioglitazone and a photoaffinity cross-linker (45). The protein identified has been termed "mitoNEET" and is located in the mitochondrial fraction of rodent...
rapid rise in ROS levels (detected 1 h after glitazone addition) reported here further argue against a key function for PPARγ in mediating cytotoxicity of glitazones in astroglial cells. Such a short time course does not seem to be compatible with the mechanism of action of this nuclear hormone receptor, namely, the regulation of gene transcription.

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