

Peroxisome Proliferator-Activated Receptor- γ Ligands Reduce Neuronal Inducible Nitric Oxide Synthase Expression and Cell Death *In Vivo*

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Expression of the inducible form of nitric oxide synthase (iNOS) in brain may contribute to neurotoxicity in Alzheimer's disease (AD). Expression of iNOS can be induced in cerebellar granule cells (CGCs) *in vivo* as well as *in vitro*, allowing these cells to be used to study regulation of neuronal iNOS expression. We report here that microinjection of bacterial lipopolysaccharide and interferon gamma into rat cerebellum induced iNOS expression in CGCs and subsequent cell death assessed by staining for DNA fragmentation. Co-injection of three structurally distinct agonists of the peroxisome proliferator-activated receptor gamma (PPAR γ), including the antidiabetic thiazolidinedione troglitazone, the non-steroidal anti-inflammatory drug (NSAID) ibuprofen, and the pro-

stanoid 15-deoxy- $\Delta^{12,14}$ prostaglandin J₂, reduced both iNOS expression and cell death, whereas co-injection of the selective cyclo-oxygenase inhibitor NS-398 had no effect. These data demonstrate that PPAR γ agonists can modulate inflammatory responses in brain. Because sustained medication with NSAIDs reduces the risk and delays the onset of AD, these results further suggest that NSAIDs provide therapeutic value by binding to PPAR γ present in AD brain, thereby preventing iNOS expression and neuronal cell death.

Key words: iNOS; PPAR γ ; cerebellar granule neurons; NSAIDs; Alzheimer's disease; apoptosis

Inflammatory activation of neuronal, as well as glial cells is believed to contribute to cell death and damage during neurological disease. In Alzheimer's disease (AD), inflammatory responses include transcription factor NF κ B activation (Kuner et al., 1998; Kaltschmidt et al., 1999) and cytokine expression near plaques (Aisen and Davis, 1994). Accumulating data (Minc-Golomb et al., 1994; Sato et al., 1995; Heneka et al., 1998) indicates that neurons can express the inducible nitric oxide synthase (iNOS), whose production of NO can be neurotoxic (Dawson et al., 1994; Skaper et al., 1995). iNOS expression can cause neuronal apoptosis *in vivo* (Matsuoka et al., 1999; Quan et al., 1999) and induce apoptosis in macrophages (Sarih et al., 1993), astrocytes (Hu and Van, 1996), and differentiated PC12 cells (Heneka et al., 1998) *in vitro*. A role for iNOS in AD is suggested by findings that neurons within tangles (Vodovotz et al., 1996) and around Hirano bodies (Lee et al., 1999) express iNOS and that staining for nitrotyrosine is increased near lesion sites (Smith et al., 1997). Suppression of neuronal iNOS expression may therefore reduce neuronal damage in AD.

Recently it was shown that activation of the peroxisome proliferator-activated receptor gamma (PPAR γ) reduces proinflammatory cytokine and iNOS expression in macrophages (Lemberger et al., 1996a; Colville-Nash et al., 1998; Ricote et al., 1998), microglial cells (Petrova et al., 1999), and monocytes (Jiang et al., 1998; Combs et al., 2000). PPAR γ is a member of the nuclear hormone receptor superfamily implicated in adipocyte differentiation, insulin sensitivity, and inflammatory processes (Lemberger et al., 1996b; Vamecq and Latruffe, 1999). The anti-inflammatory actions of PPAR γ are activated by structurally distinct ligands, including NSAIDs (Lehmann et al., 1997), antidiabetic thiazolidinediones (TZDs) (Thieringer et al., 2000), and 15-deoxy- $\Delta^{12,14}$ -prostaglandin J₂ (15d-PGJ₂), a naturally occurring agonist (For-

man et al., 1995; Kliewer et al., 1995). Activated PPAR γ heterodimerizes with the retinoic acid receptor, binds to PPAR response elements, and induces gene transcription. The anti-inflammatory actions of PPAR γ are not attributable to inhibition of cyclooxygenase (Colville-Nash et al., 1998; Heneka et al., 1999; Combs et al., 2000; Willson et al., 2000) but may be mediated by suppression of transcription factor activity (Colville-Nash et al., 1998; Ricote et al., 1999).

Recently, PPAR γ agonists were shown to protect neuroblastoma cells against neurotoxic effects of conditioned media from monocytes stimulated with β -amyloid (Combs et al., 2000) or with lipopolysaccharide (LPS) and cytokines (Klegeris et al., 1999). Because NSAID treatment reduces the risks and delays the onset of AD (McGeer et al., 1996; Stewart et al., 1997), these results suggest that PPAR γ activation by NSAIDs may mediate their therapeutic effects. Because neurons express iNOS in AD, it is possible that suppression of neuronal inflammation also contributes to the beneficial effects of NSAIDs. We recently demonstrated that iNOS expression in cerebellar granule cells (CGCs) induces cell death, which was blocked by selective iNOS inhibitors (Heneka et al., 1999) and by three structurally diverse PPAR γ ligands (Fig. 1). In the present study we demonstrate that these ligands also down-regulate neuronal iNOS expression and CGC death *in vivo*.

MATERIALS AND METHODS

Materials. LPS (*Salmonella typhirium*), phenylmethylsulfonyl fluoride, aprotinin, leupeptin, and ibuprofen (IBU) were from Sigma (St. Louis, MO); 15-deoxy- $\Delta^{12,14}$ prostaglandin J₂ (15d-PGJ₂) was from Alexis (San Diego, CA); NS-398 was from Calbiochem (San Diego, CA); and troglitazone (Trog) was a gift of Parke-Davis (Ann Arbor, MI).

Animals. Male Sprague Dawley rats (Charles River Laboratories, Wilmington, MA) weighing 250–300 gm, were housed in groups of four under standard conditions at 22°C and a 12 hr light/dark cycle with *ad libitum* access to food and water.

Injection of immunostimulants. Rats were anesthetized with pentobarbital (50 mg/kg, i.p.; dissolved in 0.9% sodium chloride) and placed on a heating blanket. Body temperature was monitored by a rectal probe connected to the heating blanket and maintained at 37 \pm 0.5°C for the time of surgery. Thereafter animals were placed in a stereotaxic frame. After exposure of the skull, a hole was drilled at the injection site, and 2 μ l of a mixture containing recombinant rat interferon gamma (IFN- γ) (20 U; Life Technologies, Gaithersburg, MD), bacterial endotoxin LPS (10 μ g; *Salmonella typhirium*; Sigma), and indicated anti-inflammatory agents (either

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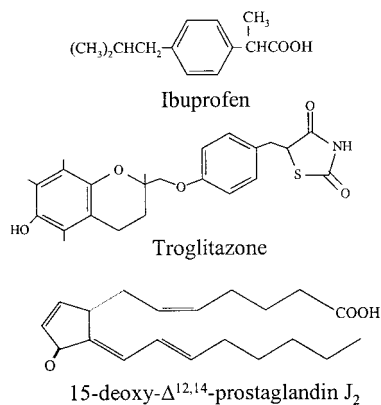


Figure 1. Structural relationship of PPAR γ ligands used in this study.

none, 100 nmol of troglitazone, 100 nmol of ibuprofen, 10 nmol of 15d-PGJ₂, or 10 nmol of NS-398) in PBS, pH 7.4, were injected over a period of 120 sec into cerebellum using a 5 μ l Hamilton syringe, at anteroposterior (AP) -12.5 , lateral (L) 0.0 , and ventral (V) 5.0 mm relative to bregma (Paxinos et al., 1985). Controls received 2 μ l of PBS. The needles were left in place for a further 5 min to prevent reflux up the needle tract. To maintain constant body temperature, animals were placed under a heating lamp until complete recovery from anesthesia. Twenty-four hours after intracerebellar injection, animals were killed by an overdose of pentobarbital and then perfused transcardially with 200 ml of heparinized sodium chloride (0.9%) and 200 ml of fixative containing 10% formaldehyde, 10% acetic acid, and 80% methanol. Brains were removed, immersed in fixative for 72 hr at room temperature, then paraffin-embedded. In some cases brains were removed without perfusion, and protein lysates were prepared for immunoblot detection. All experiments were performed in accordance with the declaration of Helsinki and the animal welfare guidelines and laws of the United States of America and were approved by the local ethical committee for animal experiments.

Processing of brain for immunohistochemistry. Immunohistochemistry was performed as previously described (Heneka et al., 2000). Serial coronal sections of the cerebellum were cut 8- μ m-thick using a Leitz microtome and mounted on poly-L-lysine-coated slides. Slides were immersed in 10 mM citrate buffer, pH 6.0, and heated in a microwave oven, four cycles of 5 min each, to unmask antigen sites. Slides were cooled for 20 min at room temperature, then washed in PBS. Endogenous peroxidase activity was inhibited by rinsing slides in 0.1% hydrogen peroxide for 10 min. Nonspecific binding was blocked by 10% normal goat serum in PBS for 1 hr at room temperature. After washing in PBS, sections were incubated overnight at 4°C with primary antibodies: (1) mAb N32020 directed against iNOS (1:200 dilution; Transduction Laboratories, Lexington, KY); (2) mAb MCA 341 raised against rat brain NOS (bNOS) (1:500 dilution; Serotec, Raleigh, NC). Sections were washed extensively with PBS, then incubated with biotinylated anti-rabbit or anti-mouse IgG (1:200 dilution; Vector Laboratories, Burlingame, CA) for 30 min at room temperature. Immunohistochemical localization was performed using the avidin–biotin peroxidase complex method (ABC kit; Vector Laboratories) with 3,3'-diaminobenzidine as chromogen.

Quantification of immunohistochemistry. Quantitative analysis of iNOS and terminal deoxynucleotidyl transferase-mediated biotinylated UTP nick end labeling (TUNEL)-positive cells was performed on brain sections from animals (three from each group). Antigens were detected in five sections having a defined distance relative to the level of cerebellar injection. The sections were the middle section corresponding to the level of injection, with the injection site discernible, and the other four sections

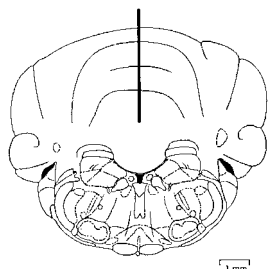
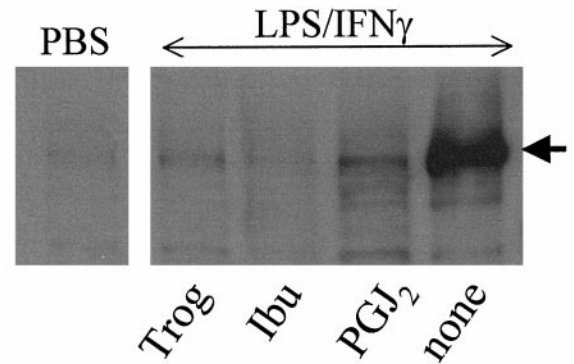
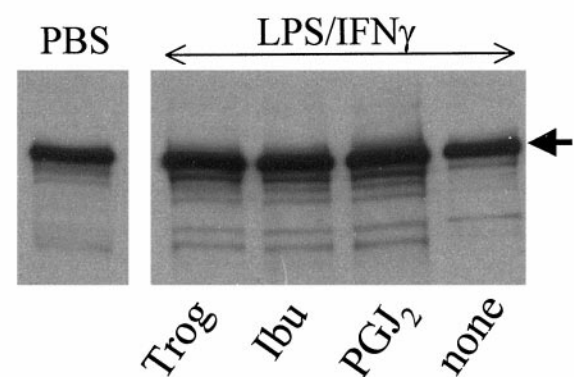


Figure 2. Schematic of injection site into cerebellum. The diagram is of a coronal section showing the position of the microinjection cannula in the rat cerebellum. The injection cannula was placed stereotaxically into the cerebellum at AP -12.5 , L 0.0 , and V 5.0 mm relative to bregma (Paxinos et al., 1985). The scale given applies in both horizontal and vertical directions.

A) iNOS



B) bNOS



PPAR- γ agonist

Figure 3. Suppression of cerebellar iNOS expression by PPAR γ agonists. A mixture of LPS (10 μ g) plus IFN- γ (20 U) dissolved in PBS was microinjected into rat cerebellum alone (none) or with ibuprofen (Ibu), troglitazone (Trog), or 15d-PGJ₂ (PGJ₂). Injection of PBS only served as control (PBS). Levels of iNOS (A) and bNOS (B) were determined 24 hr after injections by Western blot analysis. The blots shown are representative of two independent experiments.

were taken at a distance of 25 and 50 μ m rostral and caudal of the injection site. Three different areas were defined and evaluated: caudal, and left and right from the injection side. The number of cells within the respective fields was determined using a counting grid.

Western blot analysis. Protein extracts were prepared by sonication of whole brain cerebellum (25 mg of wet weight tissue) in 10 volumes of 8 M urea. Aliquots were immediately mixed with SDS sample buffer, boiled, and either used immediately or frozen at -80°C . Twenty micrograms of protein were separated through 10% polyacrylamide SDS gels, and proteins were transferred by semidry blotting to polyvinylidene difluoride membranes. The membranes were blocked in Tris-buffered saline with 0.05% Tween 20 containing 0.5% BSA, washed, incubated with primary antibodies to iNOS (mAb, 1:1,000 dilution; Cayman, Ann Arbor, MI) or bNOS overnight at 4°C, washed extensively, incubated with peroxidase conjugated goat anti-mouse IgG, and then bands were visualized with enhanced chemiluminescence reagents (Pierce, Rockford, IL).

TUNEL staining. For TUNEL staining, slides were deparaffinized, washed three times with PBS, and preincubated with 0.1 M sodium cacodylate (TDT) buffer for 5 min. Thereafter, the slides were exposed for 10 min to the reaction mixture (50 U of terminal transferase, 10 nM biotin-dUTP, and 25 mM cobalt chloride in TDT buffer). The reaction was stopped by incubating the slides for 10 min with 0.1 M sodium acetate. After blocking with 2% BSA, slides were incubated for 5 min with streptavidin–alkaline–phosphatase conjugate and developed with 0.41 mM nitroblue tetrazolium chloride and 0.38 mM 5-bromo-4-chloro-3-indolyl phosphate in 200 mM Tris-HCl, pH 9.5, containing 10 mM MgCl₂. Technical controls were done in the absence of cobalt chloride.

Protein content determination. Protein concentration was determined spectrophotometrically in 96 well plates with Bradford reagent using bovine serum albumin as standard (Bradford, 1976).

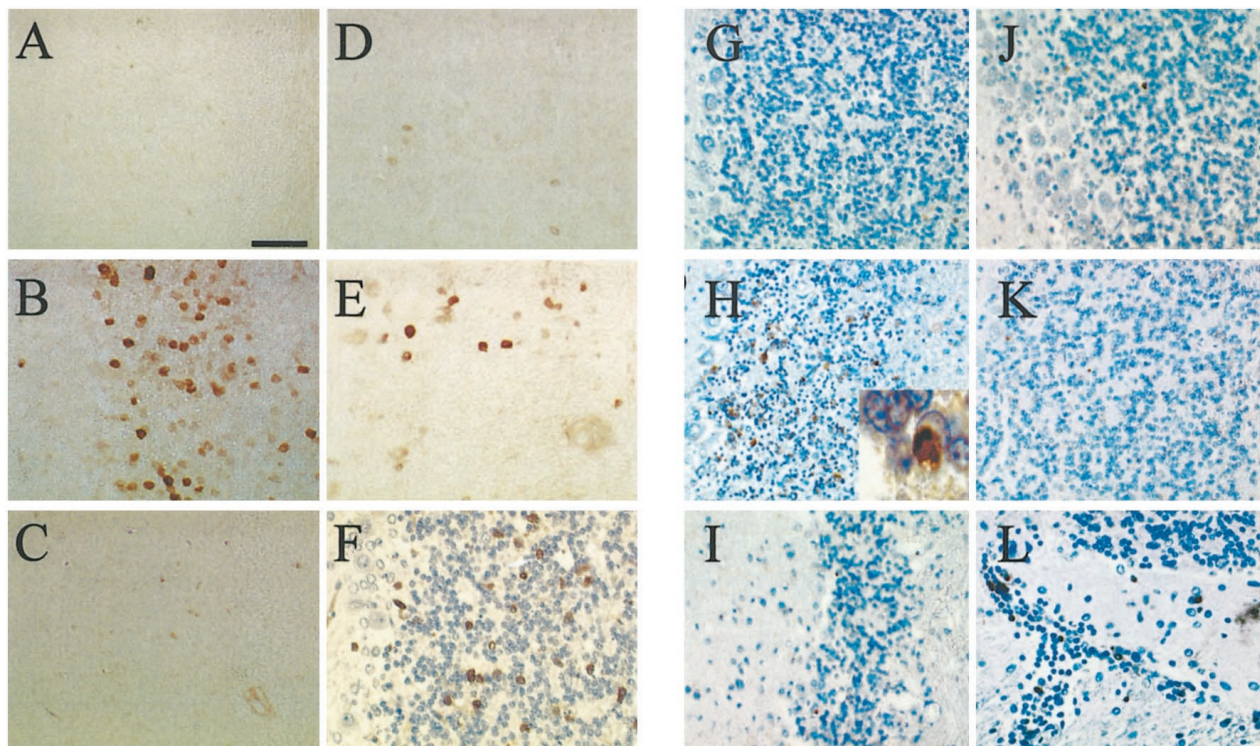


Figure 4. Immunohistochemical localization of iNOS expression and DNA strand breaks. PBS (*A, G*) or LPS plus IFN- γ in PBS (*B–F, H–L*) were injected into rat cerebellum, together with ibuprofen (*C, I*), troglitazone (*D, J*), 15d-PGJ₂ (*E, K*), or NS-398 (*F, L*). After 24 hr the brains were removed and prepared for immunohistochemical detection of either iNOS (*A–F*) or DNA breaks by the TUNEL method (*G–L*). Scale bar, 50 μ m.

Statistical analysis. Data are shown as mean \pm SD of the number of positive cells per square millimeter. Differences between controls, immunostimulated, and treated animals were assessed by one-way ANOVA followed by a Tukey test (Systat, Evanston, IL).

RESULTS

Microinjection of a mixture of LPS and IFN- γ into the vermis of the rat cerebellum (Fig. 2) induced expression of iNOS, as assessed by immunoblot analysis for iNOS protein 24 hr after injection (Fig. 3*A*). Injection of PBS served as control and failed to induce iNOS expression. Co-injection of three structurally distinct PPAR γ ligands (Ibu, Trog, and 15d-PGJ₂) reduced LPS/IFN- γ -induced iNOS expression (Fig. 3*A*). Expression of bNOS, the constitutive NOS isoform expressed by CGCs, was neither affected by immunostimulation nor by co-injection with any of the PPAR γ agonists (Fig. 3*B*).

Immunocytochemical staining of rat cerebella 24 hr after injection of LPS/IFN- γ revealed that the iNOS protein was primarily derived from CGCs (Fig. 4) and not from glial cells or macrophages. Consistent with the immunoblot results, iNOS-positive cells were not detectable after PBS injection (Fig. 4*A*). Injection of LPS/IFN- γ induced iNOS immunoreactivity almost exclusively in the granule cell layer of the cerebellum (Fig. 4*B*) and only occasionally in the molecular layer (data not shown). Immunostimulation in the presence of ibuprofen (Fig. 4*C*), troglitazone (Fig. 4*D*), or 15d-PGJ₂ (Fig. 4*E*) significantly decreased iNOS-positive staining. Immunostimulation in the presence of the cyclooxygenase (COX)-2-selective inhibitor NS-398 did not reduce iNOS-positive staining (Fig. 4*F*). For quantitative assessment of iNOS-immunopositive cells, cerebellar sections with a defined distance rostral and caudal to the level of injection were evaluated. The number of iNOS-positive cells was maximal at the level of injection (Fig. 5*A*). Ibuprofen was the most potent inhibitor and reduced iNOS-positive cell staining to background (PBS-injected) values at all distances from the injection site ($p > 0.05$ vs noninjected values; Fig. 5*B*). Troglitazone reduced overall iNOS staining across all sections by $\sim 75\%$ (from 70 ± 4 to 16 ± 1 positive cells per square millimeter per section), whereas 15d-PGJ₂ was least effective and

reduced overall iNOS staining by $\sim 50\%$ (to 31 ± 5 positive cells per square millimeter per section). In all cases the number of bNOS-expressing cells was unaffected by immunostimulation or by treatment with PPAR γ agonists (data not shown). The presence of NS-398 had no significant effect on iNOS-positive staining.

To evaluate the consequences of iNOS induction in CGCs, TUNEL staining was performed in adjacent cerebellar sections to provide an index of cell damage or death. Injection of PBS did not result in TUNEL labeling of CGCs (Fig. 4*G*). In contrast, induction of iNOS expression was paralleled by appearance of TUNEL-positive granule cells and appearance of chromatin condensation (Fig. 4*H*). As seen for iNOS staining, the number of TUNEL-positive cells was maximal at the level of injection (Fig. 5*A*) and markedly decreased in animals co-injected with either ibuprofen (Fig. 4*I*), troglitazone (Fig. 4*J*), or 15d-PGJ₂ (Fig. 4*K*). Across all sections examined, ibuprofen and troglitazone comparably reduced TUNEL-positive staining (to $\sim 10\%$ of maximal values), whereas 15d-PGJ₂ reduced TUNEL staining to $\sim 20\%$ of maximal values. The degree of TUNEL-positive staining was not affected by immunostimulation in the presence of NS-398 (Fig. 4*L*).

DISCUSSION

Induction of iNOS expression in CGCs both *in vitro* and *in vivo* has been previously described (Minc-Golomb et al., 1994; Sato et al., 1995; Heneka et al., 1999). We previously demonstrated that immunostimulation of CGCs *in vitro* results in transcription and expression of iNOS, subsequent release of NO, and induction of NO-dependent apoptotic cell death. Furthermore, we demonstrated that agonists of the PPAR γ downregulate iNOS, thereby protecting CGCs from LPS and cytokine-induced cell death *in vitro* (Heneka et al., 1999). Because CGCs express the PPAR γ *in vivo* (Braissant et al., 1996), those results prompted us to test the consequences of iNOS induction *in vivo* and the possible modulation by agonists of PPAR γ . Injection of bacterial LPS and IFN- γ into the vermis of the rat cerebellum induced iNOS expression in CGCs detected by immunoblot analysis and immunohistochemistry. In contrast, expression of bNOS, the constitutive isoform of

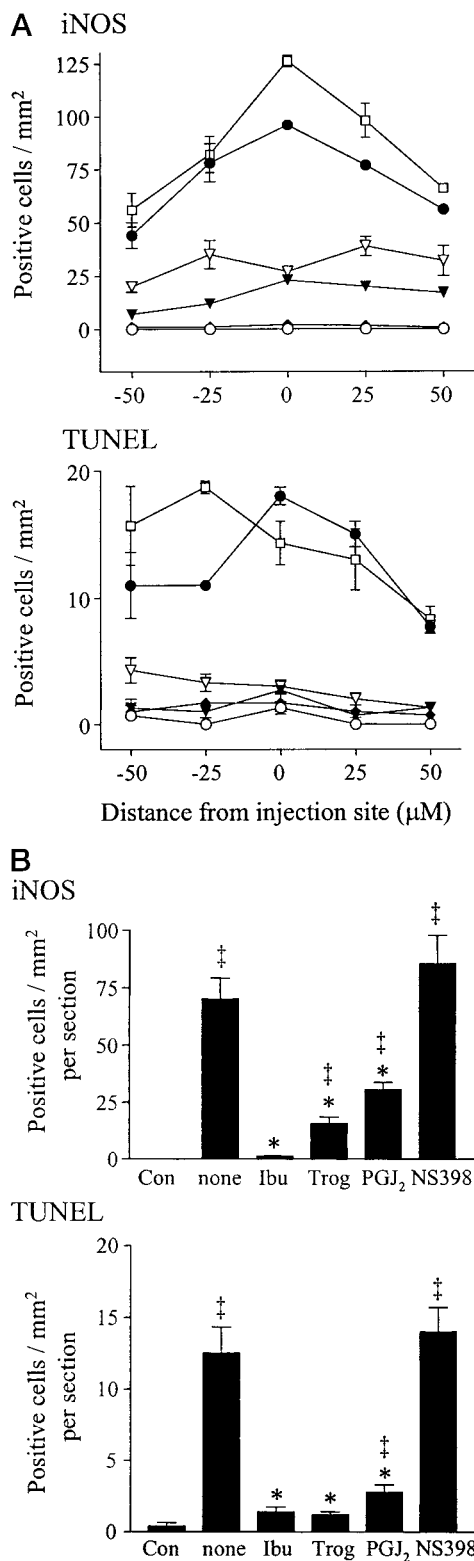


Figure 5. Quantitative analysis of iNOS expression and DNA strand breaks. *A*, Coronal cerebellar sections with a defined distance rostral and caudal to the level of injection were evaluated for iNOS-immunopositive or TUNEL-positive cells. Positive cells were counted using a counting grid and are given as positive cells per square millimeter. PBS injection (○) and immunostimulation with LPS plus IFN- γ (●) were compared to immunostimulation done in the presence of ibuprofen (◆), troglitazone (▼), 15d-PGJ₂ (▽), or NS-398 (□). For all drugs except NS-398, significance was $p < 0.01$ versus immunostimulation alone ($n = 3$ for each group). *B*, The data in *A* were analyzed as the average number of positive staining cells per square millimeter per section. * $p < 0.001$ versus immunostimulation alone (*none*); † $p < 0.01$ versus control brain (no immunostimulation) ($n = 15$ for each group).

NOS enzymes expressed by granule cells, was not affected by immunostimulation. Total iNOS protein and the number of iNOS-expressing granule cells were markedly reduced by three structurally distinct PPAR γ agonists. These results suggest that PPAR γ agonists provide similar anti-inflammatory actions *in vivo* as *in vitro*.

Our results demonstrate that increased CGC iNOS expression is accompanied by an increase in TUNEL-positive staining cells, suggesting that *in vivo*, as *in vitro*, iNOS-derived NO has neurotoxic consequences. This is supported by our findings that the decrease in iNOS expression caused by co-injection of PPAR γ ligands with LPS and cytokines is accompanied by a parallel decrease in the appearance of TUNEL-positive staining cells. However, because PPAR γ agonists can decrease expression of other cytokines, it is likely that other mechanisms contribute to their neuroprotective actions *in vivo*. Because the TUNEL method provides an index of DNA fragmentation and does not unequivocally distinguish between apoptotic versus necrotic pathways that can both lead to DNA damage, we cannot conclude if injection of LPS and cytokines causes induction of CGC apoptosis or necrosis. However, our previous findings that immunostimulation of primary CGC cultures resulted in iNOS expression, NO-dependent caspase-3 activation, DNA fragmentation, and CGC cell death suggests that at least a portion of the TUNEL-positive staining cells observed *in vivo* are undergoing apoptosis. Finally, although our data does not rule out that cells other than CGCs also show DNA damage, the fact that virtually all TUNEL staining is prevented by ibuprofen or troglitazone suggests that these drugs can act at multiple cell types.

Our *in vivo* data complements and extends recent *in vitro* studies describing anti-inflammatory actions of PPAR γ agonists in human monocytes (Klegeris et al., 1999; Combs et al., 2000). In these studies, the neurotoxic effects of conditioned media from β -amyloid or LPS plus IFN γ -stimulated human THP-1 monocytes on human neuroblastoma cells were reduced when the THP-1 cells were stimulated in the presence of NSAIDs, troglitazone, or 15d-PGJ₂, suggesting that PPAR γ ligands could reduce neurotoxicity by blocking microglial inflammatory activation. Our observations that both iNOS expression and DNA fragmentation was induced in CGCs suggests that in this model of inflammation, the neuroprotective effects of PPAR γ ligands are mediated neuronally, although effects on surrounding glial cells are not ruled out.

The exact mechanisms by which 15d-PGJ₂, ibuprofen, and troglitazone reduce iNOS expression are not yet clear. With respect to pharmacological specificity and selectivity, although the above drugs are agonists of PPAR γ , these agents may have additional actions in the brain. The identification of 15d-PGJ₂ as an endogenous ligand of PPAR γ (Kliwer et al., 1995) suggested that, at least in some cases, 15d-PGJ₂ may be acting via PPAR γ activation. This was clearly demonstrated by findings that 15d-PGJ₂ reduced iNOS expression in RAW macrophages (which did not express PPAR γ) if they were first transfected with a PPAR γ expression plasmid (Ricote et al., 1998). However, the recent demonstration that 15d-PGJ₂ (and other cyclopentenones including PGA₁) directly inhibit the activity of the I κ B kinase IKK β (Rossi et al., 2000), which is necessary to target I κ B proteins for degradation, provides a mechanism by which 15d-PGJ₂ can block NF κ B activation in the absence of PPAR γ . This may account for the ability of 15d-PGJ₂ to block iNOS expression in microglial cells, despite lack of activation of PPAR gene transcription (Petrova et al., 1999).

The TZD troglitazone has also been shown to exert potent anti-inflammatory effects on cells. Troglitazone decreased tumor necrosis factor- α synthesis and expression in phorbol myristyl acetate-activated human monocytes (Jiang et al., 1998), and ciglitazone (a closely related TZD) blocked iNOS expression in rat astrocytes (Kitamura et al., 1999b). Activation of PPAR γ by troglitazone reduced transcription from numerous promoter elements including NF κ B, GAS, AP1 (Ricote et al., 1998), and NFAT (Yang et al., 2000), which can all contribute to activation of inflammatory gene expression. Although the TZDs were developed to be high-affinity, PPAR subtype-selective agonists, with binding affinities in

the submicromolar range (Willson et al., 2000), and there are as yet no data to suggest binding to other proteins, troglitazone was reported to block iNOS expression in microglial cells without activating a PPAR-responsive reporter gene (Petrova et al., 1999). Thus, anti-inflammatory actions of troglitazone may, as the case for 15d-PGJ₂, be mediated by mechanisms in addition to PPAR γ activation.

The NSAIDs are known inhibitors of COXs, raising the possibility that the protective effects we observed after co-injection of ibuprofen were caused by inhibition of brain COX activity or expression. To address this possibility, we directly tested the effects of the selective COX-2 inhibitor NS-398 and found that this inhibitor neither reduced iNOS-positive nor TUNEL-positive staining. This finding is consistent with our previous *in vitro* studies in which we showed that concentrations of NSAIDs sufficient to inhibit COX and block prostaglandin synthesis were without effect on iNOS expression or CGC death (Heneka et al., 1999). Similarly, the neuroprotective effects of ibuprofen and indomethacin were not attributable to COX inhibition because their actions were not replicated by NS-398 (Klegeris et al., 1999; Combs et al., 2000), and neither 15d-PGJ₂ nor troglitazone have been shown to inhibit COX activity (Colville-Nash et al., 1998; Fujiwara et al., 1998). These findings suggest that the therapeutic actions of NSAIDs in AD are mediated via mechanisms other than COX inhibition, consistent with the fact that the therapeutic effects NSAIDs occur at concentrations greater than those that inhibit COX (Lehmann et al., 1997; Jiang et al., 1998) and that the COX inhibitor aspirin does not exert protective effects in AD (Stewart et al., 1997).

Observations of iNOS expression in tangle-bearing neurons (Vodovotz et al., 1996), in hippocampal neurons (Lee et al., 1999), and of increased nitrotyrosine-labeling of lesion sites in Alzheimer's disease (Smith et al., 1997) suggests that these results may have direct clinical implications. Stimulation of PPAR γ by NSAIDs has been suggested to account for the beneficial effects observed in the treatment of rheumatoid arthritis at plasma drug concentrations substantially higher than required to inhibit cyclooxygenase (Breitner et al., 1995). The epidemiological observation that long-term treatment of patients suffering from rheumatoid arthritis with NSAIDs results in reduced risk and delayed onset of AD (McGeer et al., 1996; Stewart et al., 1997), and the finding that PPAR γ is expressed in neurons (Braissant et al., 1996) and increased in AD (Kitamura et al., 1999a) suggests that PPAR γ could play a pivotal role in the pathophysiology of neurodegenerative diseases. The beneficial effects of PPAR γ agonists demonstrated in this *in vivo* study suggest that such compounds may have neuroprotective and anti-inflammatory properties. Because thiazolidinedione drugs act as PPAR γ -agonists and currently are in clinical use as antidiabetic drugs, these compounds should be considered as candidates for clinical trials in AD and neuroinflammatory disease.

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