Liver X receptor β protects dopaminergic neurons in a mouse model of Parkinson disease

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Parkinson disease (PD) is a progressive neurodegenerative disease whose progression may be slowed, but at present there is no pharmacological intervention that would stop or reverse the disease. Liver X receptor β (LXRβ) is a member of the nuclear receptor super gene family expressed in the central nervous system, where it is important for cortical layering during development and survival of dopaminergic neurons throughout life. In the present study we have used the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model of PD to investigate the possible use of LXRβ as a target for prevention or treatment of PD. The dopaminergic neurons of the substantia nigra of LXRβ$^{-/-}$ mice were much more severely affected by MPTP than were those of their WT littermates. In addition, the number of activated microglia and GFAP-positive astrocytes was higher in the substantia nigra of LXRβ$^{-/-}$ mice than in WT littermates. Administration of the LXR agonist GW3965 to MPTP-treated WT mice protected against loss of dopaminergic neurons and of dopaminergic fibers projecting to the striatum, and resulted in fewer activated microglia and astroglia. Surprisingly, LXRβ was not expressed in the neurons of the substantia nigra but in the microglia and astroglia. We conclude that LXR agonists may have beneficial effects in treatment of PD by modulating the cytotoxic functions of microglia.

midbrain | neurodegeneration | neuroinflammation

Parkinson disease (PD) is a common neurodegenerative disorder whose clinical features include tremor, slowness of movement, stiffness, and postural instability (1). PD is characterized by microgliosis, astrogliosis, progressive degeneration of dopaminergic neurons, presence of Lewy bodies in dopaminergic neurons, and α-synuclein accumulation in substantia nigra pars compacta (2). Although there are drugs that alleviate symptoms of PD, chronic use of these drugs results in debilitating side effects (3), and none seems to halt the progression of the disease. The etiology of PD remains unknown, but environmental toxins, genetic factors, and mitochondrial dysfunction are thought to be involved. Neuroinflammation (microglial activation, astrogliosis, and lymphocyte infiltration) results in production of cytotoxic molecules (4–9) that are directly involved in neuronal degeneration. It is now recognized that targeting neuroinflammation is one intervention that can slow down the progression of PD (10).

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a neurotoxin that targets rather specifically dopaminergic neurons that are involved in PD; its administration leads to severe and irreversible PD-like syndrome in humans and nonhuman primates, with most of the biochemical and pathological hallmarks of PD (11), i.e., marked loss of dopaminergic neurons, astrogliosis, and activated microglia in the substantia nigra pars compacta (12). In 2002, Wu et al. (13) showed that dopaminergic neurons in the substantia nigra could be protected from MPTP-induced damage by the tetracycline derivative minocycline. This capacity of minocycline was not due to its antibiotic activity but to its ability to suppress microglial activation. Activated microglia in their capacity as macrophages in the brain secrete a wide variety of cytotoxic agents that can cause collateral damage, i.e., kill normal neurons adjacent to neurons damaged by MPTP.

Liver X receptors (LXRα and LXRβ) are members of the nuclear receptor superfamily of ligand-activated transcription factors. These receptors are activated by naturally occurring oxysterols (14, 15). There are two synthetic LXR agonists, T0901317 and GW3965. T0901317 has been demonstrated to have agonistic effects on receptors other than LXR, such as the Farnesoid X receptor and the Pregnane X receptor (16). However, GW3965 has an agonistic effect specifically on LXR. Activation of LXRs leads to release of associated corepressor proteins and interaction with coactivators, resulting in target gene activation (17–19). LXRα, which is expressed primarily in adipose tissue, liver, and intestine, plays an important role in cholesterol homeostasis, whereas LXRβ (20–22) has key functions in the CNS and the immune system.

We have previously shown that LXRβ expression is involved in formation of superficial cortical layers and migration of later-born neurons in embryonic mice (23, 24), and that LXRβ is essential for maintenance of motor neurons in the spinal cord and dopaminergic neurons in the substantia nigra (25, 26). The substantia nigra and motor neurons in the spinal cords of LXRβ$^{-/-}$ mice are normal until the mice are 6 mo of age. After this, mice begin to perform poorly on a rotor rod, and lose the large motor neurons in the spinal cord and dopaminergic neurons in the substantia nigra (26). LXR agonists reduce inflammation by inhibiting the expression of inflammatory mediators such as inducible nitric oxide synthase, cyclooxygenase-2 and interleukin-6 in macrophages, microglia, and astrocytes (27, 28). An LXR agonist that reduces microglia activation and T-cell infiltration has been shown to have anti-inflammatory effects in experimental animal autoimmune disease (EAE) (29). The actions of another nuclear receptor, peroxisome proliferator-activated receptor (PPAR), have been studied in MPTP-induced PD and in the EAE model of multiple sclerosis. PPAR agonists were found to prevent neuroinflammation in EAE mice and to prevent the activation of MPTP to its toxic metabolites (30, 31).

The present study was designed to determine whether LXR agonist has any potential use in treatment of PD. With the use of the MPTP mouse model of PD, we show that LXRβ deficiency in mice increases MPTP-induced dopaminergic neurotoxicity, and that in WT mice an LXR agonist can slow down MPTP-induced neurodegeneration of dopaminergic neurons by inhibiting glial activation.

Results

LXRβ Mutation Aggravates the MPTP-Induced Loss of Dopaminergic Neurons in the Substantia Nigra. To evaluate alterations of dopaminergic neurons in substantia nigra, we examined the expression of tyrosine hydroxylase (TH), the rate-limiting enzyme in dopamine synthesis. We used 8-wk-old male mice for the study because at this age the substantia nigra of LXRβ$^{-/-}$ mice shows no pathology and the mice perform normally on a rotor rod. As

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expected, in both WT and LXRβ$^{-/-}$ mice, there were numerous TH-positive neurons in the substantia nigra (Fig. 1 *A–D* and *I*). Upon treatment of WT and LXRβ$^{-/-}$ littermates with MPTP, there was a decrease in the number of TH-immunopositive neurons in the substantia nigra pars compacta in both WT and LXRβ$^{-/-}$ mice (Fig. 1 *E*, *F*, and *I*). The loss was much more substantial in LXRβ$^{-/-}$ mice (Fig. 1 *G–I*). These results indicate that the presence of LXRβ limits the damage caused by MPTP.

GW3965 Protects Against the Neurodegeneration Induced by MPTP in WT Mice. After MPTP intoxication, we treated WT mice with GW3965 or vehicle for 7 d. We evaluated the alterations in the number of dopaminergic neurons in substantia nigra pars compacta. When WT mice were treated with GW3965, the MPTP-induced loss of dopaminergic neurons was less than in vehicle-treated mice (Fig. 2 *A–F* and *M*). In addition, in MPTP-treated mice, the fibers of dopaminergic neurons in the substantia nigra that project to the striatum could no longer be detected by immunoreactivity for TH (Fig. 2 *H* and *K*). These fibers were strongly stained in untreated WT mice (Fig. 2 *G* and *J*), and administration of GW3965 to MPTP-treated mice attenuated the loss of fiber staining (Fig. 2 *I* and *L*).

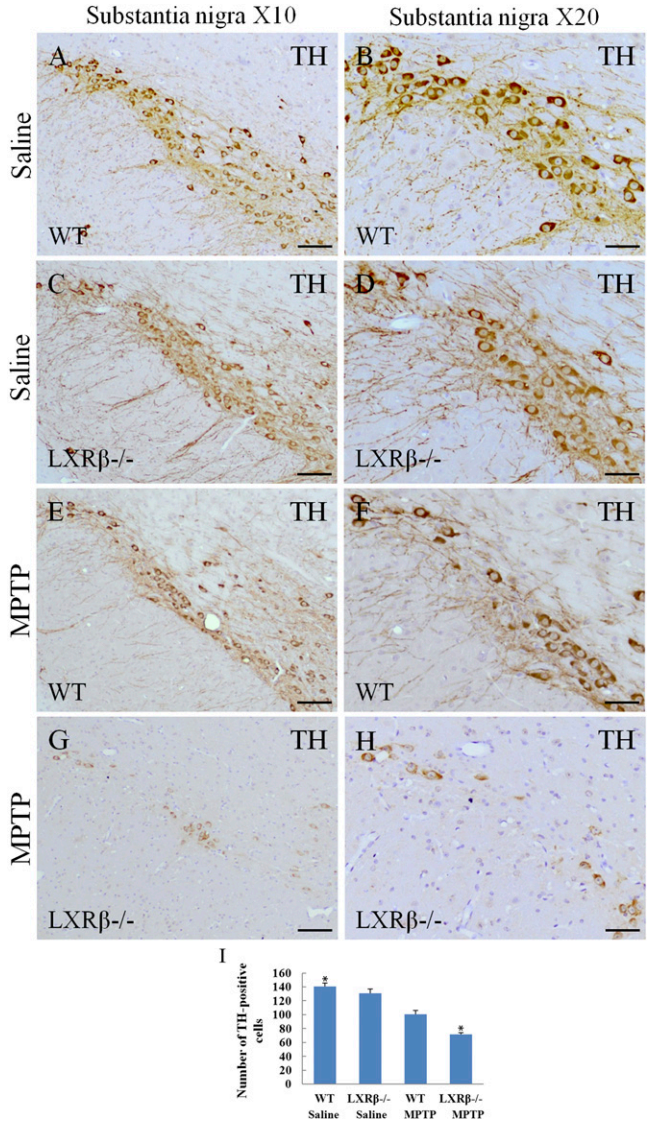

Fig. 1. TH expression in the substantia nigra of LXRβ$^{-/-}$ and WT mice treated with MPTP. (*A–D* and *I*) In WT and LXRβ$^{-/-}$ mice treated with saline, there are numerous TH-positive neurons in substantia nigra ($P > 0.05$). (*E*, *F*, and *I*) After MPTP treatment, there was a decrease in the number of TH-positive neurons in the substantia nigra (*$P < 0.01$ vs. WT mice treated with saline). (*G–I*) The loss of TH-immunopositive neurons was greater in LXRβ$^{-/-}$ mice treated with MPTP (*$P < 0.01$ vs. WT treated with MPTP). (*I*) Number of TH-positive dopaminergic neurons in substantia nigra pars compacta (WT mice treated with saline, $n = 5$; LXRβ$^{-/-}$ mice treated with saline, $n = 3$; WT mice treated with MPTP, $n = 4$; LXRβ$^{-/-}$ mice treated with MPTP, $n = 3$). (Scale bars: *A*, *C*, *E*, and *G*, 100 μm; *B*, *D*, *F*, and *H*, 50 μm.)

LXRβ Is Expressed in Glial Cells, Not in Neurons of Substantia Nigra. We used a specific antibody, which has been well characterized for staining of LXRβ in the brain (22), to detect expression of LXRβ in the substantia nigra of WT mice. LXRβ was expressed in the nuclei of glial cells of both substantia nigra pars compacta and pars reticularis (Fig. 3*A*). No LXRβ staining was detectable in the neurons of either pars compacta or pars reticularis (Fig. 3 *B* and *C*). The specificity of the antibody for LXRβ was evident from staining of the brains of LXRβ$^{-/-}$ mice: no LXRβ could be detected either in glia or in neurons (Fig. 3 *D–F*).

LXRβ Mutation Augments Glial Reaction in Substantia Nigra. Microglia are the resident innate immune cells in CNS. The resting microglia are small cells with long and thin ramified processes. In the substantia nigra of both WT and LXRβ$^{-/-}$ mice, there were a few microglial (Iba1-positive) cells with small cell bodies and long and thin ramified processes in Fig. 4 *A*, *B*, *E*, *F*, and *Q*. In WT mice intoxicated with MPTP, the resting microglia became activated, as evidenced by larger cell bodies and poorly ramified, short, and thick processes. In addition, the number of microglia (Iba1-positive) was increased in substantia nigra pars compacta (Fig. 4 *C*, *G*, and *Q*). Treatment of LXRβ$^{-/-}$ mice with MPTP resulted in accumulation of many more activated microglia in substantia nigra pars compacta than was observed in WT mice treated with MPTP (Fig. 4 *D*, *H*, and *Q*). In untreated WT and LXRβ$^{-/-}$ mice, GFAP (astrocyte marker) was mainly expressed in substantia nigra pars reticularis, with few GFAP cells in pars compacta (Fig. 4 *I*, *J*, *M*, *N*, and *R*). After MPTP intoxication, GFAP expression was increased in both pars compacta and reticularis of WT mice (Fig. 4 *K*, *O*, and *R*). In LXRβ$^{-/-}$ mice treated with MPTP, there were more activated astrocytes with more astrocytic projections in substantia nigra (Fig. 4 *L*, *P*, and *R*).

GW3965 Reduces the Activation of Glial Cells in Substantia Nigra of MPTP-Treated WT Mice. When MPTP-intoxicated WT mice were treated with GW3965 or with DMSO (the vehicle for GW3965; Fig. 5 *B*, *E*, and *M*), there were fewer Iba1-positive cells in substantia nigra pars compacta in the GW3965-treated group, and these Iba1-positive cells had small cell bodies with long and thin ramified processes (Fig. 5 *C*, *F*, and *M*). As expected in normal mice that were not challenged with MPTP, there were a few Iba1-positive resting microglia in substantia nigra and no activated microglia (Fig. 5 *A* and *D*).

Scattered GFAP-positive cells (astrocytes) were located in substantia nigra pars reticularis of WT control mice (Fig. 5 *G* and *J*), and after MPTP intoxication, the number of GFAP-positive cells increased. Furthermore, after MPTP treatment, in both pars compacta and pars reticularis, these astrocytes were activated, as evidenced by the increased number of ramified processes (Fig. 5 *H*, *K*, and *N*). Treatment of MPTP-intoxicated mice with GW3965 resulted in an attenuation of the increase in GFAP-positive cells in substantia nigra pars compacta (Fig. 5 *I*, *L*, and *N*). These results show that as was the case for microglia, GW3965 treatment reduced the activation of astrocytes in the MPTP mouse model.

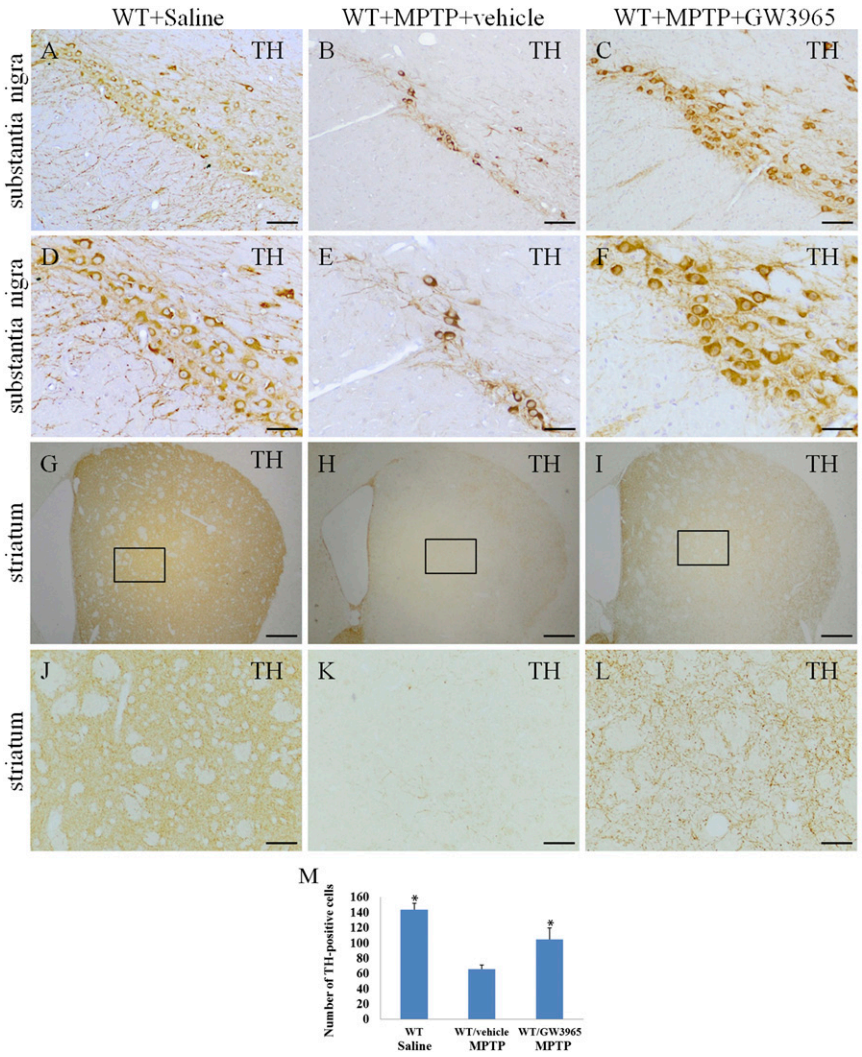

Fig. 2. TH expression in the substantia nigra and striatum of MPTP-intoxicated WT mice treated with GW3965 or vehicle. (*A*, *B*, *D*, *E*, and *M*) The number of TH-immunopositive neurons decreased in WT mice treated with MPTP (*$P < 0.01$ vs. WT mice treated with saline). (*C*, *F*, and *M*) In GW3965-treated mice, dopaminergic neurons were protected from MPTP toxicity (*$P < 0.01$ vs. MPTP-WT treated with vehicle). (*G* and *J*) The fibers in the striatum of WT mice treated with saline were intensely stained. (*H* and *K*) MPTP injection led to an overall reduction of TH expression in the striatum in WT mice treated with vehicle. (*I* and *L*) GW3965 treatment reduced the MPTP-induced loss of TH-positive fibers in the striatum. (*M*) Number of TH-positive dopaminergic neurons in substantia nigra pars compacta (WT mice treated with saline, $n = 5$; MPTP WT mice treated with vehicle, $n = 4$; MPTP WT mice treated with GW3965, $n = 4$). *D–F* and *J–L* are magnified views for *A–C* and *G–I*, respectively. (Scale bars: *A–C*, 100 μm; *D–F* and *J–L*, 50 μm; *G–I*, 200 μm.)

Discussion

Previous research has demonstrated lipid deposition, gliosis, and degeneration of neurons in the substantia nigra of aged LXR double-knockout animals (32), whereas LXRβ$^{-/-}$ mice develop ALS–Parkinson-like syndrome after 6 mo of age. These observations suggest that LXRβ may be protective against neurodegeneration of substantia nigra (25). Furthermore, there is activation of microglia in the substantia nigra of aging LXRβ$^{-/-}$ mice, and this suggests that LXR may have immunosuppressive effects in the brain (26) as it does in the rest of the immune system. The present study demonstrates that LXRβ$^{-/-}$ mice treated with MPTP lost more TH-positive neurons in the substantia nigra and had more activated microglia and GFAP^{+} astrocytes in this part of the brain. Thus, LXRβ deficiency in mice increased the susceptibility to the neurotoxin MPTP. Treatment of MPTP-intoxicated WT mice with the LXR agonist GW3965 attenuated the loss of TH-positive neurons in the substantia nigra and TH-positive fibers in the striatum, and there were fewer activated microglia and astrocytes in the substantia nigra. Thus, GW3965 can attenuate the MPTP-induced loss of dopaminergic neurons and glial activation.

With the use of a specific LXRβ antibody, we found that LXRβ is expressed in both substantia nigra pars compacta and pars reticularis, and that the nuclear staining was localized in the glia of substantia nigra, not in neurons. The presence of LXRβ in glial cells supports the idea that LXR could play a role in neuroinflammation. According to the present study, LXRβ$^{-/-}$ mice treated with MPTP lost more TH-positive neurons than did WT mice, with more glial activation in the substantia nigra. Thus, the reduction in degeneration of dopaminergic neurons caused by GW3965 administration appears to occur not through a direct

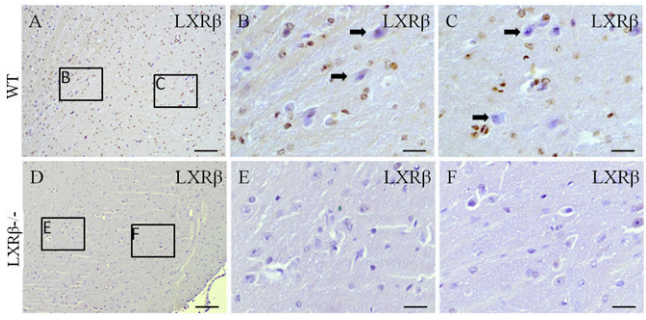

Fig. 3. Immunohistochemical study of the expression of LXRβ in substantia nigra of mice. (*A*) LXRβ was expressed in both substantia nigra pars compacta and pars reticularis. LXRβ-positive staining was in the nuclei of glial cells of the substantia nigra. (*B* and *C*) The neurons in both pars compacta and pars reticularis were completely LXRβ negative. The black arrows point to the LXRβ-negative neurons. (*D–F*) In the LXRβ$^{-/-}$ mice, LXRβ could not be detected in glia or in neurons. (Scale bars: *A* and *D*, 100 μm; *B*, *C*, *E*, and *F*, 20 μm.)

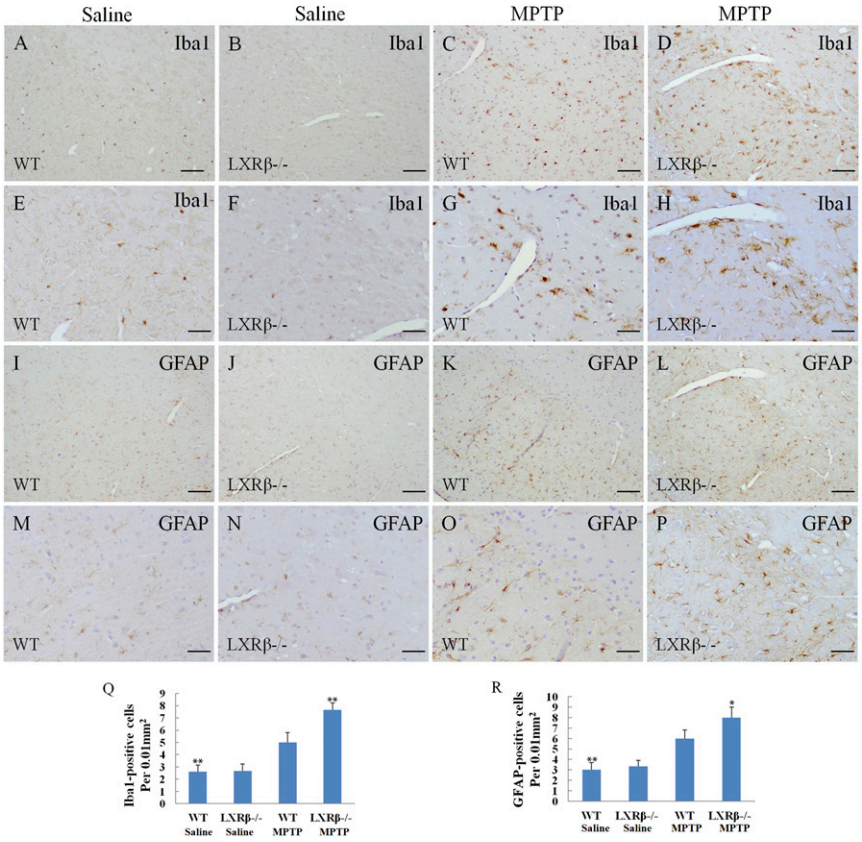

Fig. 4. Expression of microglia (Iba1) and astrocytes (GFAP) in substantia nigra of WT and LXRβ$^{-/-}$ mice treated with MPTP. (*A*, *B*, *E*, *F*, and *Q*) In WT and LXRβ$^{-/-}$ mice treated with saline, there were a few Iba1-positive cells with small cell bodies and long and thin ramified processes in substantia nigra ($P > 0.05$). (*C*, *G*, and *Q*) After treatment of WT mice with MPTP, the number of Iba1-positive cells increased (**$P < 0.01$ vs. WT mice treated with saline), and these cells had larger cell bodies and poorly ramified short and thick processes in substantia nigra pars compacta. (*D*, *H*, and *Q*) In LXRβ$^{-/-}$ mice treated with MPTP, there were more activated microglia in substantia nigra pars compacta than in WT mice treated with MPTP (**$P < 0.01$ vs. WT treated with MPTP). (*I*, *J*, *M*, *N*, and *R*) GFAP was mainly expressed in substantia nigra pars reticularis of WT and LXRβ$^{-/-}$ mice treated with saline, with a few positive cells in the pars compacta. (*K*, *O*, and *R*) After MPTP intoxication, GFAP expression was increased in both pars compacta and reticularis of WT mice (**$P < 0.01$ vs. WT mice treated with saline). (*L*, *P*, and *R*) In LXRβ$^{-/-}$ mice treated with MPTP, there were more activated astrocytes with more processes in substantia nigra (*$P < 0.05$ vs. WT treated with MPTP). (*Q*) Density of Iba1-positive microglia in substantia nigra. (*R*) Density of GFAP-positive astrocytes in substantia nigra (WT mice treated with saline, $n = 5$; LXRβ$^{-/-}$ mice treated with saline, $n = 3$; WT mice treated with MPTP, $n = 4$; LXRβ$^{-/-}$ mice treated with MPTP, $n = 3$). *E*–*H* and *M*–*P* are magnified views for *A*–*D* and *I*–*L*, respectively. (Scale bars: *A*–*D* and *I*–*L*, 100 μm; *E*–*H* and *M*–*P*, 50 μm.)

effect on dopaminergic neurons but through the glia. Thus, the protection conferred by LXR and its agonists is different from that observed for PPAR, which appeared to prevent metabolic activation of MPTP. Because the LXR agonist was administered after the mice had received four doses of MPTP, it is unlikely that the activation of MPTP to its toxic metabolite was involved.

Use of LXR agonists has been reported to up-regulate α-synuclein expression in human neuroblastoma (SH-SY5Y) cells. Such induction would be expected to have deleterious effects on dopaminergic neurons in PD (33), because accumulation of α-synuclein, the major component of Lewy body inclusions, increases fibrillization, thereby contributing to neurodegeneration (34–36). In SH-SY5Y cells, 24-hydroxycholesterol and 27-hydroxycholesterol, which are ligands for LXR, have differential effects on tyrosine hydroxylase and α-synuclein: 24-hydroxycholesterol increases the levels of tyrosine hydroxylase, whereas 27-hydroxycholesterol increases the levels of α-synuclein (37). 27-Hydroxycholesterol activates LXRβ and induces its binding to LXRE in the α-synuclein promoter, up-regulating α-synuclein expression in SH-SY5Y cells (33, 38). In these in vitro studies, LXRβ was expressed in neuroblastoma cells. However, in vivo, we found no immunostaining of LXRβ in neurons of the substantia nigra in adult mice. Because the LXRβ antibody has been extensively tested in the brain where it stains neurons in the fetal and neonatal brain (23, 24), we are confident in our finding that LXRβ is not expressed in adult neurons, and conclude that an LXR agonist may not directly regulate the expression of α-synuclein in dopaminergic neurons in vivo. However, because nuclear receptors can be modified post-transcriptionally, we cannot exclude the possibility that some modification of the N terminus of LXRβ (which was the site of the epitope used in raising the antibody) has occurred in the mature neurons that leads to loss of the ability of the antibody to recognize the receptor. To address this issue we have also tried a commercially available antibody (GTX89661; GeneTex) that recognizes epitopes in the N terminus of LXRβ; however, with this antibody, results were similar to those obtained with the one raised in our laboratory.

Microglia, the macrophage-like resident glia, are the innate immune cells in the CNS (39). Under neuropathological conditions, microglia are rapidly activated in response to neuronal damage. Microglia and astrocytes are thought to be the primary sources of deleterious chemokines and cytokines that participate in neuroinflammation. Both these cell types exhibit a reactive phenotype in association with neurodegenerative disease. It is currently thought that innate immunity significantly contributes to dopaminergic neurodegeneration in PD (40). Microglia are activated in PD (8), in MPTP-intoxicated patients (41), and in MPTP-induced animal models of PD. Activated microglia mediate the neuroinflammatory response, and elevated inflammatory factors lead to neurodegeneration of dopaminergic neurons in the neighborhood of those neurons that had been damaged by MPTP (8). Blocking microglial activation in the MPTP-induced model of PD does attenuate dopaminergic neurodegeneration (13). In the present study, GW3965 treatment of mice attenuated MPTP toxicity in the dopaminergic neurons of the substantia nigra. GW3965 reduced activation of microglia and astrocytes in the substantia nigra of MPTP-treated mice. Inhibition of glial activation and reduction of glial-induced neurotoxicity by GW3965 appear to have prevented the loss of dopaminergic neurons in the PD mouse model. The present study is in line with a study by Zelcer et al. (42) showing that an LXR agonist inhibits the inflammation response of primary glial cell and increases phagocytic capacity to fibrillar Aβ peptide in the setting of Alzheimer's disease in vitro.

In summary, the present study demonstrates that LXRβ mutation aggravates the MPTP-induced loss of dopaminergic neurons and activation of glial cells in the substantia nigra. GW3965 can reduce the activation of glial cells and reduce the

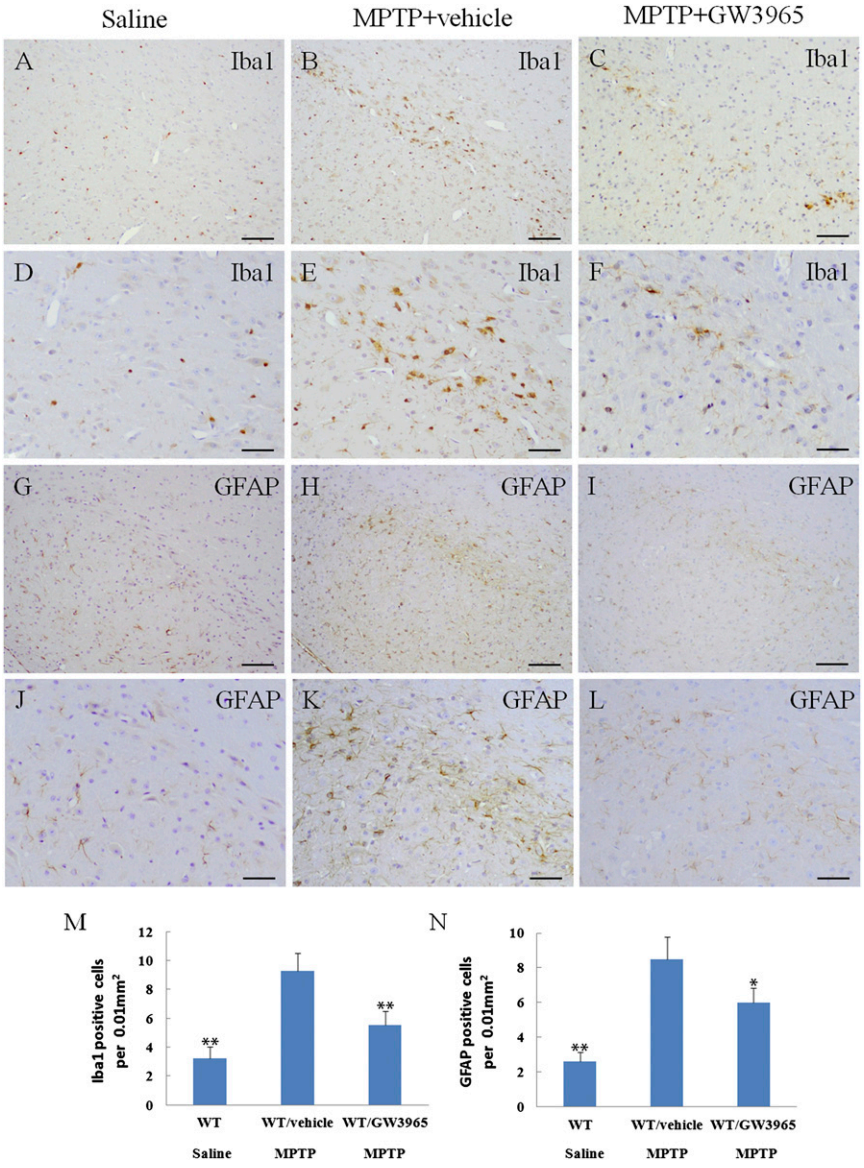

Fig. 5. Expression of Iba1 and GFAP in the substantia nigra of MPTP-intoxicated WT mice treated with GW3965 or vehicle for 7 d. (*A* and *D*) In WT mice treated with saline, there were a few Iba1-positive resting microglial cells in substantia nigra. (*B*, *E*, and *M*) After MPTP intoxication, the number of Iba1-positive cells increased (**$P < 0.01$ vs. WT mice treated with saline). These cells had larger cell bodies and poorly ramified short and thick processes in substantia nigra pars compacta. (*C*, *F*, and *M*) In the GW3965 treatment group, there were far fewer Iba1-positive cells in substantia nigra pars compacta (**$P < 0.01$ vs. MPTP WT treated with vehicle), and these Iba1-positive cells had small cell bodies and long and thin ramified processes. (*G*, *J*, and *N*) Scattered GFAP-positive cells (astrocytes) were located in substantia nigra pars reticularis of WT mice treated with saline. (*H*, *K*, and *N*) After MPTP treatment, the number of GFAP-positive cells with more processes increased in both pars compacta and reticularis (**$P < 0.01$ vs. WT mice treated with saline). (*I*, *L*, and *N*) In the GW3965 treatment group, there were fewer GFAP-positive cells in the substantia nigra pars compacta (*$P < 0.05$ vs. MPTP WT treated with vehicle). (*M*) Density of Iba1-positive microglia in substantia nigra. (*N*) Density of GFAP-positive astrocytes in substantia nigra (WT mice treated with saline, $n = 5$; MPTP WT mice treated with vehicle, $n = 4$; MPTP WT mice treated with GW3965, $n = 4$). *D–F* and *J–L* are magnified views for *A–C* and *G–I*, respectively. (Scale bars: *A–C* and *G–I*, 100 μm; *D–F* and *J–L*, 50 μm.)

MPTP-induced loss of dopaminergic neurons. Our results suggest that LXR agonists may have a role in therapeutic intervention in PD and perhaps other neurodegenerative diseases where neuroinflammation plays an important role in pathogenesis.

Materials and Methods

Materials. 1-Methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine hydrochloride (MPTP HCl) was purchased from Toronto Research Chemicals. LXR agonist GW3965 (3-[3-[*N*-(2-chloro-3-trifluoromethylbenzyl)-(2,2-diphenylethyl) amino]propyloxy]phenylacetic acid hydrochloride) was purchased from Sigma-Aldrich.

Mice. All mice were 8-wk-old males purchased from Taconic. To detect the sensitivity of LXRβ$^{-/-}$ mice to MPTP, LXRβ$^{-/-}$ mice and C57BL/6J (WT littermates) mice were treated with MPTP; WT and LXRβ$^{-/-}$ control mice were treated with saline only. To examine the use of GW3965 in the MPTP mouse model, 15 WT mice were divided randomly into three groups: (*i*) WT control; (*ii*) WT treated with MPTP and vehicle [DMSO/saline (1/10)]; (*iii*) WT treated with MPTP and GW3965. For MPTP intoxication, mice received four i.p. injections of MPTP HCl (16 mg/kg of free base; Toronto Research Chemicals) in saline at 2-h intervals, and control mice received only saline (13). GW3965 was dissolved in DMSO/saline (1/10); GW3965 (20 mg/kg) was administered s.c. daily for 7 d starting 3 h after the last MPTP injection (43). Mice were housed in a room of standard temperature (22 ± 1 °C) with a regular 12-h light/12-h dark cycle and given free access to water and standard rodent chow. All animal experiments were carried out according to the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (44) and approved by the National Animal Experiment Board, Finland. To detect the sensitivity of LXRβ$^{-/-}$ mice to MPTP, mice were killed 48 h after the last injection of MPTP. To evaluate the effect of GW3965 on the MPTP mouse model, mice were killed 24 h after the last GW3965 treatment. All mice were terminally anesthetized by pentobarbital (60 mg/kg Mebunat; Orion Pharma) and transcardially perfused with heparinized (2.5 IU/mL) saline followed by 4% (wt/vol) paraformaldehyde in 0.1 M PBS (pH 7.4). All brains were dissected and postfixed in the same fixative overnight at 4 °C. After fixation, brains were processed for paraffin sections (5 μm).

Immunohistochemistry. Paraffin sections were deparaffinized in xylene, rehydrated through graded alcohol, and processed for antigen retrieval by boiling in 10 mM citrate buffer (pH 6.0) for 2–3 min. Sections were incubated in 3% H_2O_2 in PBS for 20 min at room temperature to quench endogenous peroxidase. To block nonspecific binding, sections were incubated in 3% BSA for 20 min, and then a biotin blocking system (Dako) was used to block endogenous biotin. Sections were then incubated with anti-TH (1:100; Santa Cruz Biotechnology), anti-Iba1 (1:400; Abcam), anti-GFAP (1:400; Santa Cruz Biotechnology), and anti-LXRβ (1:1,000; made in

Jan-Ake Gustafsson's laboratory) at 4 °C after blocking nonspecific binding in 3% BSA. BSA replaced primary antibodies in negative controls. After washing, sections were incubated with goat HRP polymer kit (Biocare Medical; GHP516) for 30 min at room temperature, followed by 3,3-diaminobenzidine tetrahydrochloride as the chromogen (45, 46). The number of TH-positive neurons of the substantia nigra in each mouse was counted under light microscopy as described previously (47–49).

Data Analysis. Data are expressed as mean ± SD. Statistical comparisons were made using a one-way ANOVA followed by a Newman–Keuls post hoc test. $P < 0.05$ was considered to indicate statistical significance.

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