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Protective properties afforded by pioglitazone against intrastriatal LPS in Sprague-Dawley rats

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Abstract

We created an inflammation-induced Parkinson's disease model, where microglia activation leads to oxidative stress, mitochondrial dysfunction, and dopaminergic neurodegeneration in the substantia nigra. Pioglitazone, an agonist of peroxisome proliferator activated receptor- γ (PPAR- γ), can prevent these deficits and protect dopaminergic neurons. To continue exploring the effects of pioglitazone in this model we focused on the expression of PPAR- γ , uncoupling protein 2 (UCP2), and mitoNEET. We report that intrastriatal lipopolysaccharide (LPS) increases striatal PPAR- γ , UCP2, and mitoNEET expression, and pioglitazone attenuates these LPS-induced changes.

Keywords

Parkinson's disease; PPAR- γ ; UCP-2; mitoNEET; inflammation

INTRODUCTION

Chronic inflammation plays a role in the pathogenesis of Parkinson's disease (PD) [25], and several lipopolysaccharide (LPS)-induced PD models [3,19,22,30] have focused on inflammation's role in PD-related neurodegeneration. In these models, LPS activates microglia, increases oxidative stress, impairs mitochondrial function, and induces what appears to be specific dopaminergic neurodegeneration [19,22]. Pioglitazone, an agonist of peroxisome proliferator activated receptor- γ (PPAR- γ), can prevent LPS-induced dopaminergic neurodegeneration [22], and may be useful as a PD treatment [21]. However, little is known about the mechanistic effects of pioglitazone, which in addition to its anti-inflammatory properties may also affect mitochondrial uncoupling proteins [23] and bioenergetics [12,22]. Here we focus on the effects of LPS and pioglitazone on PPAR- γ , uncoupling protein 2 (UCP2), and mitoNEET expression.

PPAR- γ is one of three different PPAR isoforms that regulates gene expression using various ligand-dependent and -independent molecular processes to decrease several pro-inflammatory genes and immunoregulate microglia [5,8,11]. However, its expression was not determined in our intrastriatal LPS model [22].

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UCP2 is one of five acknowledged UCPs and it is located in the inner mitochondrial membrane, where it helps reduce the proton gradient [24]. Biologically active substances like LPS affect the transcription, translation, and/or activity of UCP2 [15]. In addition, UCP2 may be involved in PD [1,10] and PPAR- γ agonists can increase its expression [23]. For a detailed review on UCP2 and its function, see Mattiasson and Sullivan 2006 [24].

The recently described “mitoNEET” protein, named based on its localization in the outer mitochondrial membrane and the Asn-Glu-Glu-Thr (NEET) sequence in its carboxyl terminal domain, is hypothesized to be a framework upon which mitochondrial metabolism can be controlled [9,29]. It has been suggested that some effects of pioglitazone are mediated by changes in mitochondrial metabolism [12,16], and mitoNEET, which has a binding affinity for pioglitazone, might be the target site for these effects [9].

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats, age three months, were obtained from Harlan (Indianapolis, IN) and were housed under a 12hr light–dark cycle with free access to food and water. Protocols involving the animals were in strict accordance with the *Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee at the University of Kentucky.

Treatments and Injections

Animals were pretreated once a day via oral gavage, four days prior to injection, with either 0.3ml of vehicle (pure olive oil) or pioglitazone in vehicle suspension (Actos, Takeda Chemical Industries, 20mg/kg). On day five rats were intrastrially injected with either 0.9% sterile saline or LPS as previously described [22]. A pain score rating and body condition scoring ensured humane endpoints, and the rats resumed their treatments until euthanizing three days post injection.

Western Blot

Animals were euthanized, brains removed, and the striatum was dissected out. Western blot was used to analyze PPAR- γ , UCP2, and mitoNEET expression. Protein samples were normalized and prepared for loading into an appropriate gel to be run at 80 volts. The Kaleidoscope prestained marker from BioRad (Hercules, CA) served as a molecular weight marker, and gels were transferred to Nitrocellulose at 60 volts for 2h. The membrane was washed, blocked, and incubated in primary antibody (anti- β -actin - Sigma 1:4000, anti-PPAR- γ – Upstate 1:500, anti-UCP2 – Alpha Diagnostic 1:200, and anti-mitoNEET – gift from Dr. Ross 1:500) [29]. Next, the membrane was washed and incubated in an appropriate secondary antibody (1:2000) for 1h. After incubation, the membrane was washed and the ECL Plus kit and Hyperfilm (Amersham Biosciences, Little Chalfont Buckinghamshire, UK) were used for chemiluminescent detection. Loading errors were corrected by using β -actin immunoreactive bands, density measurements were performed with the Image Quant version 5.1 (Molecular Dynamics Inc.), and background was subtracted to calculate mean band density.

Statistical Analysis

The GraphPad Prism software (version 4.03, GraphPad Software, San Diego, CA) was used to perform an ANOVA followed by the Newman-Keuls multiple-comparison post hoc test. Statistical significance was set at $p < 0.05$.

RESULTS

Pioglitazone prevents the LPS-induced increase in PPAR- γ

PPAR- γ is found in the striatum [27] and its expression can be altered by LPS [12,14]. In addition, pioglitazone protects against LPS toxicity [22]. Western blot analysis showed a trend ($p=0.11$) for LPS to increase PPAR- γ . However, pioglitazone treatment significantly ($p\leq 0.05$) attenuated the apparent LPS-induced PPAR- γ increase (Figure 1).

UCP2 is increased by LPS and pioglitazone prevents this

UCP2 expression can be altered by LPS [15] and PPAR- γ agonists [23]. Western blot analysis provided evidence that LPS significantly increased UCP2 ($p\leq 0.01$) and pioglitazone ($p\leq 0.001$) prevented this increase (Figure 1).

Pioglitazone prevents the LPS-induced increase in mitoNEET

The hypothesis that mitoNEET may be an alternate target site for pioglitazone [9] led us to determine the expression level of this protein. Western blot analysis showed LPS significantly increased mitoNEET ($p\leq 0.01$) and pioglitazone restored mitoNEET levels ($p\leq 0.01$) similar to control (Figure 1).

DISCUSSION

Intrastriatal LPS significantly increased striatal UCP2 and mitoNEET expression as well as showed a trend for increased PPAR- γ , three days post injection. In addition, pioglitazone prevented the LPS-induced increase in PPAR- γ , UCP2, and mitoNEET. We hypothesize that these changes may play a role in the inflammation, oxidative stress, mitochondria dysfunction, and dopaminergic neurodegeneration following intrastriatal LPS [22], and that some of the protective properties afforded by pioglitazone may be a result of the decreased striatal PPAR- γ , UCP2, and mitoNEET expression observed here.

In this study, intrastriatal LPS showed a trend to increase PPAR- γ expression relative to control, and pioglitazone significantly reduced the LPS-induced increase in striatal PPAR- γ . LPS can increase PPAR- γ mRNA and treatment with a PPAR- γ agonist prevents this increase [14]. Therefore, a potential role for PPAR- γ mediated signaling may exist in our model. However, further validation of PPAR- γ mediated effects using other agonists as well as antagonists should be performed in the future, as the anti-inflammatory effects of pioglitazone and the agonists are observed at concentrations much higher than their EC₅₀ values for binding to or activating PPAR- γ [16].

We observed a significant increase in striatal UCP2 expression after LPS, which was prevented by pioglitazone. Support for our data, comes from the observation LPS affects the transcription, translation, and/or activity of UCP2 [15]. In addition, UCP2 may play a role in immunomodulation [4], or as a protective response to cell stress in hypermetabolic states like sepsis [15]. Therefore, since the main physiological role of UCP2 is to lower reactive oxygen species production [7,13] UCP2 expression might be increased as a compensatory response to the inflammation and oxidative stress that follows LPS injection. Support for this concept comes from evidence that suggests UCP2 is involved in neuroprotection against a PD model [1,10], where deletion of UCP2 enhanced the susceptibility of dopaminergic neurons towards cell death [2]. However, in this case UCP2 over-expression may be detrimental to the cell as exacerbated uncoupling could result in mitochondrial impairment. Evidence for over-exaggerated uncoupling being detrimental comes from the market removal of the uncoupling diet drug dinitrophenol [26]. Thus, pioglitazone may have attenuated the LPS-induced over-expression of UCP2 to provide protection against over-uncoupling. The fact that UCP2 is

altered by PPAR- γ agonists [23] supports this hypothesis. A caveat to this is UCP2 was increased by PPAR- γ agonists [18]; although, this was in non-brain tissue and LPS was not used. An alternate interpretation could be UCP2 was decreased by pioglitazone as resulted of an attenuated inflammatory response and oxidative stress. Future studies will need to determine the exact role of UCP2 in this model as well as the effects of pioglitazone on striatal UCP2 expression.

We demonstrated, for the first time, that intrastriatal LPS increases striatal mitoNEET expression and pioglitazone can prevent this increase. Although, we have no explanation for the increase in mitoNEET, we speculate that increased mitoNEET may impair the mitochondria, as LPS increased mitoNEET expression in our model at a time when mitochondrial respiration was impaired [22]. In addition, the LPS-induced increase in mitoNEET as well as proton shuttling from increased UCP2 may cause the release of mitoNEET's iron sulfur clusters, which would increase free iron and lead to mitochondrial localized oxidative stress. Thus, pioglitazone may bind to mitoNEET [9] to stabilize the iron sulfur clusters [20,28], and this would provide some of the protection against mitochondrial impairment that we previously reported [22]. This hypothesis appears to have potential validity, as mitoNEET may serve as a framework upon which mitochondrial metabolism can be controlled [9,29]. The fact that pioglitazone alters mitochondrial proteins [6] also suggests a possibility for pioglitazone to decrease mitoNEET expression. In our LPS model, it appears that mitoNEET may play some role; however, further exploration will be needed as there is much to learn about this new protein.

While we did not co-label different cell-types with these proteins, we know that mitoNEET and UCP2 are found in mitochondria, which would be in both neurons and glia. The same goes for PPAR- γ , which has been shown in microglia, dopaminergic neurons, and astrocytes. Therefore, pioglitazone probably affects striatal glia as well as the dopaminergic neuron terminals, which project from the SN to release dopamine in the striatum. However, since dopaminergic neurons appear to be preferentially susceptible to LPS-induced inflammation [19,22], striatal damage would cause retrograde degeneration as first hypothesized by Greenamyre et al. 1999 [17]. Future studies should focus on the localization of these proteins and the local effects of pioglitazone, to elucidate the exact meaning of each of these events. Overall, the modulation of PPAR- γ , UCP2, and mitoNEET expression by LPS and pioglitazone, may be involved in the degeneration and protection against intrastriatal LPS. Therefore, this study further supports the use of pioglitazone as a novel treatment for neuroinflammation and PD.

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Figure 1.

Western blot analysis shows a trend for LPS to increase PPAR- γ expression. In addition, LPS significantly increases both UCP2 and mitoNEET. Treatment with pioglitazone significantly attenuates the LPS-induced increase in PPAR- γ , UCP2, and mitoNEET (Figure 1A). Data are expressed as means $\pm$ SEM, from $n=3-5$ /group, ** or ## = $p \leq 0.01$, where ** represents Vehicle/Saline (V/S) vs. Vehicle/LPS (V/L) and ## represents Vehicle/LPS vs. Pioglitazone/LPS (P/L). Figure 1B shows representative blots for PPAR- γ , UCP2, mitoNEET, and beta-actin.