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Research Report

Dietary supplementation with resveratrol protects against striatal dopaminergic deficits produced by *in utero* LPS exposure

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ABSTRACT

The purpose of this study was to determine the effect of dietary supplementation with the anti-inflammatory compound resveratrol in pregnant dams on lipopolysaccharide (LPS)-induced dopaminergic deficits in pups exposed to LPS *in utero*. Gravid female rats were fed with a resveratrol-enriched diet during gestational days 3–22.5 (E3–E22.5) and received an intraperitoneal (i.p.) injection of 1 mg/kg LPS at E10.5. The striata were isolated from the pups at postnatal days 10 (P10) and P21. LPS-induced dopaminergic deficits were noted at P21, but not P10. These DA deficits at P21 were exhibited by a loss of DA and DA metabolite [3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA)] levels and tyrosine hydroxylase (TH) expression in the striatum. The LPS-induced loss of DA, DA metabolites, and TH expression were attenuated in the striata of pups from the dams fed with the resveratrol-supplemented diet. These data suggest that a resveratrol-supplemented diet may restore homeostasis of the striatal DA neuronal system following disruption by LPS.

1. Introduction

Lipopolysaccharide (LPS), an endotoxin found in the outer membrane of gram-negative bacteria, induces strong systemic inflammatory immune responses and release of cytokines (Okeke et al., 2013). Due in part to this immune response, gram-negative bacterial infections, such as bacterial vaginosis (BV), have been shown to cause complications in early pregnancy that may lead to fetal death in humans. In addition, gram-negative bacterial endotoxins, such as LPS, have been found to be considerably damaging to preimplantation and implantation stage embryos (Ling et al., 2002b; Deb et al., 2004; Aroutcheva et al., 2008), and the developing brain (Ling et al., 2002a, 2002b; Ghiani et al., 2011; Xu et al., 2013). In the brain, LPS causes an inflammatory response through the activation of microglia and subsequent release of proinflammatory cytokines (Laurenzi et al., 2001; Park et al., 2007; Dutta et al., 2008; Poulose et al., 2012). Besides microglia activation, LPS also causes activation of other brain cells, such as astrocyte, resulting in an inflammatory response (Waak et al., 2009).

Neuroinflammation develops after exposure to LPS (Kim et al., 2000; Ling et al., 2002b, 2004b; Dutta et al., 2008; Gao et al., 2008) and a significant decrease in DA, DA metabolites, and TH levels, a phenotypic marker of dopamine neurons, in

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the striatum (STR) have been noted in adult animals (P60) following exposure to LPS (Kirsten et al., 2010, 2012). Decrease in the levels of TH was also observed in substantia nigra (SN) in prenatal and adult animals following exposure to LPS (Liu et al., 2000; Gao et al., 2002; Ling et al., 2002a, 2002b). As an increase in chronic inflammation and a loss of DA neurotransmission are hallmarks of Parkinson's disease (PD), an early exposure to LPS has been suggested to increase susceptibility to the development of PD later in life (Gao et al., 2002; Ling et al., 2004a, 2004b; Dutta et al., 2008; Choi et al., 2011). Therefore, there is an interest in inhibiting the endotoxin-induced neurodegeneration that occurs in prenatal and adult animals.

Natural supplements that are isolated from fruits and berries have been studied as potential therapies for neuroinflammation due to their anti-inflammatory and anti-oxidant properties and widespread availability (Vauzour et al., 2008; Sebai et al., 2009). Specifically, resveratrol, a phytochemical found in peanuts and the skin of grapes, has been shown to provide neuroprotection *via* the inhibition of microglia activation and subsequent release of proinflammatory factors *in vivo* and *in vitro* (Ling et al., 2004a; Baur and Sinclair, 2006; Meng et al., 2008; Abraham and Johnson, 2009; Lu et al., 2010; Zhang et al., 2010b).

Although a role for resveratrol in neuroprotection against LPS in an adult animal model has been investigated (Sebai et al., 2009), the effect of providing a resveratrol-supplemented diet to a pregnant dam on LPS-induced toxicity in the offspring has not been examined. In the current study, we tested the hypothesis that a resveratrol-supplemented diet during gestation will inhibit LPS-induced dopaminergic deficits noted during postnatal development in the offspring exposed to LPS *in utero*. The resveratrol diet was administered from embryonic day 3 (E3), before LPS exposure at E10.5, to the end of the gestational period (E22.5). At postnatal days 10 and 21 (P10 and P21, respectively), we examined the DA and DA metabolite contents and TH and DA transporter (DAT) expressions in the striatal tissue of the offspring. Our data suggest that LPS exposure *in utero* leads to dopaminergic deficits at P21 that are alleviated by a resveratrol-enriched diet during gestation (Fig. 1).

2. Results

2.1. *LPS-induced loss of DA, DOPAC, and HVA content in the striatal tissue of P21, but not P10, pups*

HPLC was performed to measure DA, DOPAC, and HVA content and DA turnover in the striatal tissue of P10 and P21 pups. We did not observe any LPS-induced changes in striatal DA, DOPAC, and HVA content at P10 as compared to those of control (Table 1). In contrast, LPS treatment significantly decreased striatal DA, DOPAC, and HVA content at P21 as compared to those of control (Fig. 2A–C). No change in DA turnover, as measured by DOPAC/DA, HVA/DA and (DOPAC+HVA)/DA, was observed in any group (Table 1 and Fig. 2D–F).

2.2. *Dietary resveratrol supplementation during gestation protected against the LPS-induced loss of DA, DOPAC and HVA content in the striatum tissue of P21 pups*

HPLC was performed to measure DA, DOPAC, and HVA content and DA turnover in the striatal tissue of P21 pups. At P21, striatum DA, DOPAC, and HVA levels in the LPS+RV group were restored to that of the control group (Fig. 2A–C). These data suggest that resveratrol protects against an LPS-induced decrease in DA and DA metabolites in the striatum at this age.

2.3. *Dietary resveratrol supplementation during gestation reversed the LPS-induced loss of TH expression in the striatal tissue of P21 pups*

Western blot analysis was performed to determine TH expression in the striatum tissue of P10 and P21 pups. Tyrosine hydroxylase expression was decreased in the striatum following LPS exposure at P21 (Fig. 3C), but not P10 (Fig. 3A). Dietary resveratrol supplementation significantly increased TH expression in the P21 pups as compared to that of the LPS-treated group at the same age (Fig. 3C).

2.4. *Neither in utero exposure to LPS nor dietary resveratrol supplementation during gestation altered DAT expression in the striatum tissue*

Western blot analysis was performed to determine DAT expression in the striatum tissue of P10 and P21 pups. LPS, resveratrol, or LPS+RV treatment did not affect striatal DAT expression for any age (Fig. 3B and D). Together our data suggest that although LPS-induced significant losses of DA and TH content at P21, DA neuronal function, as indicated by DAT expression, was not altered in the striatum in any treatment or age group.

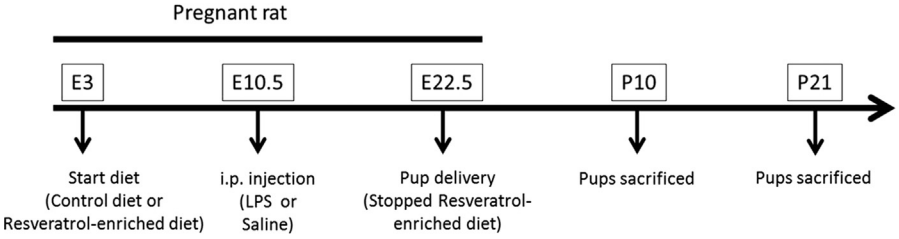

Fig. 1 – *In vivo* experimental design. Gravid females received control or resveratrol-enriched diet from E3–E22.5. They are administered an i.p. injection of either saline or LPS at E10.5. The rats deliver their pups normally at E22.5 and the pups are sacrificed at P10 and P21.