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Research Report

Short-term effects of an endotoxin on substantia nigra dopamine neurons

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ABSTRACT

Inflammation has been implicated in the pathology of several neurodegenerative diseases, including Parkinson's disease (PD). Studies using the endotoxin lipopolysaccharide (LPS), a potent inflammogen, show that systemic insults can trigger prolonged microglial activation and pro-inflammatory cytokine production leading to degeneration of substantia nigra (SN) dopamine (DA) neurons, mimicking idiopathic PD. Because rapid effects of LPS on SN neurons had not been investigated previously, the focus of this study is to assess time-dependent alterations in SN neuroinflammation, DAergic neurons, and neuronal signaling cascades following LPS administration. LPS (5 mg/kg, i.p.) or saline (0.9% NaCl) was administered to 8-month-old male mice. At 3 h, 5 h, and 12 h post-injection, the morphology of the SN was assessed using antibodies directed against tyrosine hydroxylase (TH, DAergic marker), Iba-1 (pan-microglial marker), phospho-ERK, and phospho-CREB (signaling). LPS administration significantly reduced TH-immunoreactivity (ir) at all time-points with the greatest reduction observed at 12 h post-injection. Reduced TH-ir was accompanied by a significant increase in activated microglia at all time-points following LPS. By 12 h post-injection, LPS-treated mice exhibited activated as well as reactive microglia, which can result in neuronal damage. These data demonstrate that the initial reduction in TH-ir observed after an LPS injection was not concomitant with morphological alterations in microglial cells, even though a significant increase in phospho-ERK was observed in glial cells as soon as 3 h post-injection. It is possible that the initial alteration in DA phenotype (TH reduction) may perpetuate an inflammatory response that persists and leads to further DAergic damage.

1. Introduction

Inflammation is an active defense reaction of multi-cellular organisms against diverse insults, designed to remove or inactivate noxious agents and to inhibit and reverse their detrimental effects (for review see Gao et al. (2003), Liu et al. (2003)). Microglia are responsible for producing an inflammatory reaction to insults (Streit et al., 2004). Normally, microglia

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are in a resting state characterized by a ramified morphology, and low expression levels of MHC Class II elements. However, during inflammatory changes in their micro-environment or as a consequence of pathological changes, they transform into their activated state by displaying an ameboid morphology and increased thickness and number of processes (Kreutzberg, 1996).

Microglial activation occurs in association with various brain injuries (Kato and Walz, 2000), including damage common to neurological diseases (Banati et al., 1998; Versijpt et al., 2003), and following administration of neurotoxins utilized to model neurodegenerative disorders (Finsen et al., 1993; Scali et al., 1999; Fiedorowicz et al., 2001). Microglia perform various functions in tissue repair and neural regeneration by releasing trophic factors (Nakajima and Kohsaka, 2004). However, activated microglia mainly act as scavenger cells by initiating inflammation and exacerbating degeneration causing an increased release of pro-inflammatory molecules such as reactive oxygen species (ROS), interleukin (IL)-1β, tumor necrosis factor α (TNFα), and nitric oxide (NO), the overproduction of which can cause toxicity (Kennedy et al., 1997, Hald and Lotharius, 2005).

Direct evidence of cell loss triggered by microglial activation comes from studies assessing *in vivo* effects of the gram-negative bacterial cell wall component lipopolysaccharide (LPS). LPS has been injected into the SN of rats causing microglial activation and degeneration of the dopaminergic system (Castano et al., 1998; Herrera et al., 2005). Similarly, a single systemic LPS injection in mice models has been shown to induce chronic neuroinflammation with subsequent neurodegeneration (Qin et al., 2007; Granholm et al., 2011). *In vitro* studies using mixed neuron-glia cultures treated with LPS demonstrated rapid microglial activation followed by neuronal degeneration (Gao et al., 2002). Additionally, LPS administration *in vivo* has been shown to result in reduced SN TH-immunoreactivity as well as DA content when administered either prenatally or postnatally in both mice (Liu et al., 2008; Granholm et al., 2011) and rat studies (Castano et al., 1998). These studies suggested that SN DA neurons may be particularly sensitive to inflammatory processes in the brain, but studies have not been undertaken to determine the temporal determinants of LPS-induced dopaminergic degeneration. Therefore, the aims of the current study were to examine short-term effects of systemic LPS injections on DA neuron

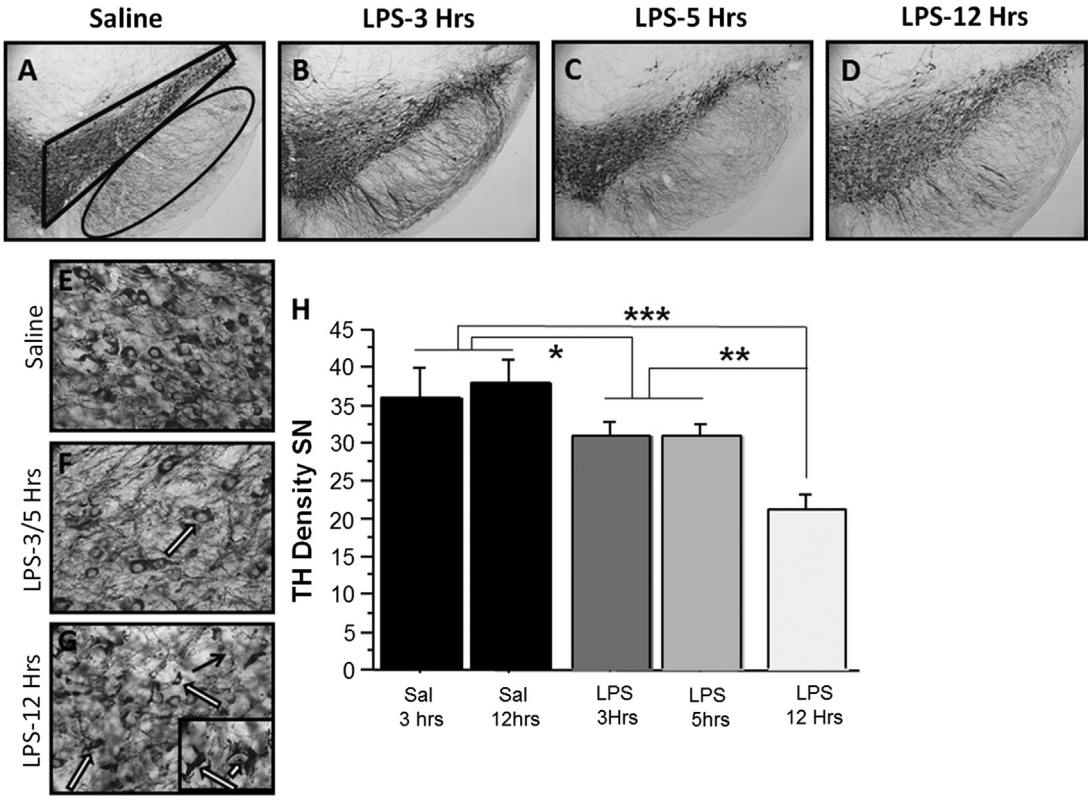

Fig. 1 – Nigral TH-ir is reduced following LPS administration. Photomicrographs of TH-ir in the SN of (A) saline-treated mice, (B) LPS-treated mice at 3 h, (C) LPS-treated mice at 5 h, and (D) LPS-treated mice at 12 h (magnification = 10 ×). (E-G) Treatment groups at magnification = 60 ×. (H) Quantification of the average TH-ir density in the SNpc. LPS-treatment, regardless of time-point, resulted in reduced TH-ir compared to saline-treatment ($p < 0.05$). No difference existed in the density of TH-ir between 3- and 5-h LPS treated mice, however, by 12 h, TH-ir was significantly reduced compared to 3- and 5-h post-injection ($p < 0.01$). At 5-h post-LPS injection, nuclear swelling was evident as indicated by the large arrow. By 12 h post-LPS injection, existing TH-ir cells are showing changes in morphology, including cellular shrinking as indicated by the large arrows and punctate processes as indicated by the thin arrow in 1F. SNpc outlined by straight lines, SNpr outlined by oval shape. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.