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Maternal exposure to nonylphenol during pregnancy and lactation induces microglial cell activation and pro-inflammatory cytokine production in offspring hippocampus

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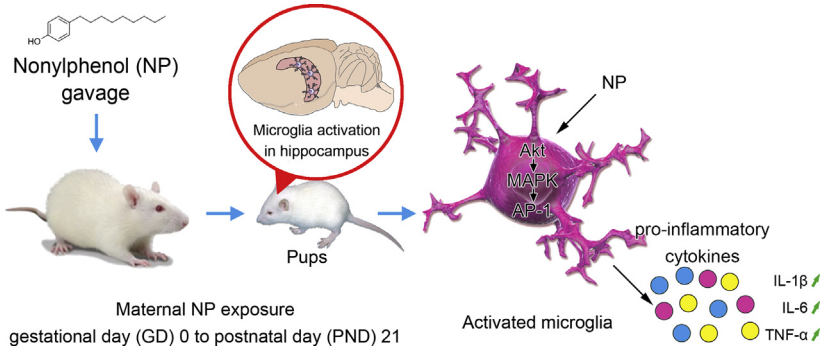
HIGHLIGHTS

- Data on effects of nonylphenol exposure during pregnancy and lactation on offspring is limited.
- Maternal exposure to nonylphenol induces microglia activation in offspring hippocampus.
- Microglia activated by nonylphenol produce excessive pro-inflammatory cytokines in offspring.
- Nonylphenol-induced pro-inflammatory cytokine production is mediated by Akt/MAPK/AP-1 signaling.

GRAPHICAL ABSTRACT



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ABSTRACT

Recently, environmental nonylphenol (NP) exposure in the fetus and child has received increasing attention because of its potentially deleterious effects on the central nervous system (CNS). Microglia (MG), resident immune cells in the CNS, are vital to CNS homeostasis and defense against exogenous chemicals, which makes them a potentially sensitive target of NP. The present study aims to explore the effects of maternal NP exposure during pregnancy and lactation on MG in offspring hippocampus, the production of pro-inflammatory cytokines by MG, and associated underlying mechanisms. We found that maternal NP exposure increased the production of interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) in offspring hippocampus. Increases in both activation and number of MG were observed in offspring hippocampus. Increased phosphorylation of Akt was found to co-localize with hippocampal MG, while increased phosphorylation of c-Jun N-terminal kinase (JNK), and p38 mitogen-activated protein kinase (MAPK) were observed in offspring hippocampus. Activator protein 1 (AP-1), an inflammatory transcription factor, was also activated in the hippocampus of pups subjected to maternal NP exposure. These results suggest that maternal NP exposure might activate MG in offspring

Abbreviations: AD, Alzheimer's disease; AP-1, activator protein 1; BBB, blood-brain barrier; BPA, bisphenol A; CNS, central nervous system; COX-2, cyclooxygenase-2; DAPI, 4′,6 diamidino 2 phenylindole; EDC, endocrine disrupting chemical; ELISA, enzyme-linked immunosorbent assay; ERK, extracellular regulated protein kinase; GD, gestational day; HD, Huntington's disease; IL-1β, interleukin-1β; IL-6, interleukin-6; iNOS, inducible nitric oxide synthase; JNK, c-Jun N-terminal kinase; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MG, microglia; MS, multiple sclerosis; NOAELs, No Observed Adverse Effect Levels; NP, nonylphenol; p-Akt, phosphorylated Akt; PB, placental barrier; PBS, phosphate buffered saline; p-ERK, phosphorylated ERK; p-JNK, phosphorylated JNK; PND, postnatal day; p-p38, phosphorylated p38; SDF-1, stromal cell-derived factor-1; SEM, standard error of mean; TBS, Tris-HCl buffered saline solution; TBST, Tris-buffered saline with Tween 20; TNF-α, tumor necrosis factor-α.

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hippocampus. This activation seems to subsequently increase the production of IL-1β, IL-6, and TNF-α. Furthermore, Akt/MAPK/AP-1 signaling may be involved in this activation of MG and increased production of pro-inflammatory cytokines.

1. Introduction

Nonylphenol (NP), an endocrine-disrupting chemical (EDC), originates principally from the degradation of nonylphenol ethoxylates, which are common industrial surfactants (Manzano et al., 1998). Unbound NP leaches into the environment from all sorts of products including textiles and plastic waste in landfills and is also discharged in sewage, causing contamination of aquatic system and sediments (Soares et al., 2008). Humans are largely exposed to NP by the intake of its contaminated food and drinking water (Uguz et al., 2003). A recent report showed that 100% of non-occupationally exposed women in Southern Spain were exposed to NP (Lopez-Espinosa et al., 2009). Moreover, the highest level of NP in human breast milk of Italian women was 32 ng/ml (Ademollo et al., 2008). The estimated mean intake of NP from food per capita was 27 μg/day (medium bound) in the general Swedish population (Gyllenhammar et al., 2012). The concentration of NP in urban eutrophic lakes in subtropical China reached 1.9–32.8 μg/l in lake water and 3.5–32.4 mg/kg in lake sediments (Wu et al., 2007). It will take quite a long time for the environment to degrade the discharged NP (Liber et al., 1999), although there are some restrictions on the use of NP products, such as the ban on NP marketing and use in the European Union (Union, 2003).

Recently, the effects of NP exposure on the central nervous system (CNS) of the fetus and child have received increasing attention, because it may result in impairments in the CNS and may interfere with CNS development (Ferguson et al., 2000). NP treatment induced apoptosis in mouse hippocampal neuronal cells in early developmental stages (Litwa et al., 2014). Chronic application of NP (100 and 200 mg/kg/day) by oral gavage activated abnormal apoptosis in young mice brain (Mao et al., 2008). After ingestion, NP circulates via the bloodstream throughout the CNS and penetrates the blood-brain barrier (BBB) (Arukwe et al., 2000). It can also penetrate placental barrier (PB) and be detected in the NP-exposed mother's milk (Ademollo et al., 2008; Bechi et al., 2010). These features of NP make CNS a potentially sensitive target during pregnancy and lactation (Woo et al., 2007). Inappropriate or harmful maternal diet during pregnancy and lactation can result in impaired neural development and behavioral disorders in the offspring (Min et al., 2016; Sullivan et al., 2014; Wang et al., 2014). Maternal chronic NP application by oral gavage was found to induce cognitive impairment in juvenile mice (Mao et al., 2010). However, research on the effects of maternal NP exposure on offspring CNS is limited. The underlying mechanisms by which impairment of offspring CNS is induced by maternal NP exposure remain to be elucidated.

The hippocampus is an important region of learning and memory in the CNS (Morris et al., 1990). There exist a large number of microglia (MG) during neural development in the hippocampus (Milligan et al., 1991). MG are resident immune cells located in the CNS and are vital to CNS homeostasis and defense against exogenous chemicals and physical changes (Tan et al., 2014). However, abnormal activation of MG and the corresponding excessive cytokine levels may cause severe damage to the CNS (Marín-Teva et al., 2011). Excessive pro-inflammation cytokines are directly produced by activated MG in the CNS. Neonatal lipopolysaccharide (LPS) exposure resulted in elevation of interleukin-1β (IL-1β) in rat hippocampus and long-lasting learning disabilities (Wang et al., 2013). Brain interleukin-6 (IL-6), a pro-inflammatory cytokine, has been found to mediate autism-like behaviors in post-mortem and animal studies (Wei et al., 2013). Neuroinflammation, marked by over-activated MG, is believed to contribute to a wide spectrum of neurodegenerative diseases such as Alzheimer's disease (AD), Huntington's disease (HD), and multiple sclerosis (MS) (Klegeris et al., 2007).

Complex signaling cascades are involved in the activation of MG (Brough et al., 2002; Wilms et al., 2003). Previous studies provided evidence that the mitogen-activated protein kinase (MAPK) signaling can be initiated by phosphorylated Akt and can regulate inflammatory responses in MG (Park et al., 2011). An inhibitor of c-Jun N-terminal kinase (JNK) reduced bisphenol A (BPA)-induced MG inflammatory responses (Zhu et al., 2015). In addition, p38 mitogen-activated protein kinase (MAPK) plays an important role in cytokine production, which has been implicated in AD (Obata et al., 2000). Upregulation of extracellular signal-related kinases (ERK) is found in stromal cell-derived factor-1 (SDF-1)-induced MG activation and IL-6 production (Lu et al., 2009). Activator protein 1 (AP-1) is regarded as a key downstream nuclear transcription factor of the MAPK signaling for the inflammatory response (Silvers et al., 2003) and was activated in LPS-induced production of pro-inflammatory cytokines in BV2 MG (Luo et al., 2016).

In this study, offspring rats were subject to NP exposure by performing maternal intragastric administration of NP during pregnancy and lactation. We sought to explore the effects of maternal NP exposure during pregnancy and lactation on MG in the hippocampus including the production of pro-inflammatory cytokines. The Akt/MAPK/AP-1 signaling may be involved in the activation of MG and their production of pro-inflammatory cytokines. Therefore, in the current study, we also investigated whether Akt/MAPK/AP-1 signaling is involved in mediating the effects of maternal NP exposure on MG and the production of pro-inflammatory cytokines.

2. Materials and methods

2.1. Reagents and antibodies

4 *n* Nonylphenol (purity > 99%) was acquired from Acros Organics (New Jersey, USA; Fig. 1A). Modified AIN-93G diet (a low phytoestrogen diet with 7% corn oil substituted for 7% soybean oil) was purchased from HFK Bioscience Co., Ltd. (Beijing, China; Fig. 1B). Corn oil purchased from SIGMA (St. Louis, USA) was used as NP delivery vehicle. Rat IL-1β, IL-6, and TNF-α ELISA kits were obtained from eBioscience (Vienna, Austria). RIPA lysis buffer, ECL (Enhanced Chemiluminescence) Plus reagent, and the BCA Protein Assay kit were purchased from Beyotime (Shanghai, China). The AlexaFluor 594-conjugated goat anti-rabbit-IgG secondary antibody (1:200) was purchased from Life Technologies (Rockford, IL, USA) and Alexa Fluor 488-conjugated goat anti-mouse-IgG secondary antibody (1:200) was purchased from Invitrogen (Rockford, IL, USA).

2.2. Animals

Female and male Wistar rats (240–260 g) were obtained from the Center of Experimental Animals at China Medical University (Shenyang, China) with a National Animal Use License number of SCXK-LN2003-0009. Animal use was approved by the Animal Use and Care Committee at China Medical University. All experiments and surgical procedures were approved by the Animal Use and Care Committee at China Medical University, which complies with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Whenever possible, we sought to minimize the number of animals used and their suffering.