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The dopamine D3 receptor regulates the effects of methamphetamine on LPS-induced cytokine production in murine mast cells

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ABSTRACT

Previous studies have demonstrated that methamphetamine (METH) alter inflammatory and anti-inflammatory cytokine production in the periphery. However, the effect of METH on lipopolysaccharide (LPS)-induced immune responses and its underlying mechanism of action remains unclear. The dopamine D3 receptor (D3R) plays an important role in METH addiction, indicating that the D3R may regulate METH-mediated immune responses. In this study, we examined the effect of METH on mast cell released cytokines in the lungs and thymi of mice stimulated by LPS, and on LPS-induced murine bone marrow-derived mast cells (BMMCs). Moreover, we used D3R-deficient mice to investigate the effect of this receptor on LPS-stimulated mast cell released cytokine production after METH treatment in the lungs and thymi. The effects of a D3R agonist and antagonist on LPS-induced cytokine production after METH treatment in murine BMMCs were also evaluated. METH suppressed LPS-induced cytokine production in the lungs and thymi of wild-type (WT) mice and BMMCs. However, METH did not alter LPS-induced cytokine production in the lungs and thymi of D3R-deficient mice. When BMMCs were treated with the D3R receptor antagonist, NGB2904 hydrochloride (NGB-2904), METH did not alter LPS-induced cytokine production. However, treatment with the D3R agonist, 7-hydroxy-(di-*n*-propylamino) tetralin (7-OH-DPAT), significantly enhanced the effects of METH on LPS-induced cytokine production. Our results suggest that METH regulates mast cell released cytokines production in an LPS-induced mouse model *via* the D3R.

Introduction

Methamphetamine (METH) is a powerful stimulant with potent addictive and neurotoxic properties. METH abuse has become a public health problem in recent years, with approximately 35 million abusers worldwide (Hamamoto and Rhodus, 2009). Recent studies have shown that METH abuse is associated with an increased risk of human immunodeficiency virus (HIV) infection, hepatitis B and C infections, and other diseases (Halkitis et al., 2001; Gonzales et al., 2006; Miller et al., 2009). Emerging studies have demonstrated that METH can alter the production of both inflammatory and anti-inflammatory cytokines in the periphery (Yu et al., 2002a,b; In et al., 2005; Hozumi et al., 2008). METH also causes immune dysfunction in mature mammals by negatively altering antibody and cytokine production in mice (Martinez et al., 2009). Furthermore, METH significantly alters cellular mediators in the lungs (Peerzada et al., 2013). Lipopolysaccharide (LPS), a component of the outer membrane of gram-negative bacteria and a commonly used immunostimulant, mediates the alterations in central and peripheral tissues

Abbreviations: METH, methamphetamine; HIV, human immunodeficiency virus; D3R, dopamine D3 receptor; DA, dopamine; LPS, lipopolysaccharide; TNF-a, tumour necrosis factor; TLR4, Toll-like receptor 4; NSn, ormal saline; BMMC, bone marrow-derived mast cells; WT, wild-type; SCF, stem cell factor; 7-OH-DPAT, 7-hydroxy-(di-*n*-propylamino) tetralin (DPAT) hydrobromide; NGB2904, NGB2904 hydrochloride; ELISA, enzyme-linked immunosorbent assay.

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associated with gram-negative infections (Singh and Jiang, 2004). It was reported that METH administration exacerbated the expression of pro-inflammatory cytokines, including tumour necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6 following LPS stimulation in mouse brains (Buchanan et al., 2010). However, how METH regulates these cytokines in peripheral tissues after LPS stimulation remains unclear.

Mast cells are immune sentinel cells that directly respond to pathogens, thereby modulating both innate and adaptive immune responses. In particular, mast cells play an important role in the lungs (Moiseeva and Bradding, 2011; Campillo-Navarro et al., 2014). Recent studies have also shown that thymic mast cells play a significant role during infection-induced thymus involution and thymus-related diseases (Marinova et al., 2007; Raica et al., 2007). Furthermore, mast cells can modulate host innate immune responses through the release of cytokines (Abraham and St John, 2010; Dawicki and Marshall, 2007). LPS-mediated Toll-like receptor 4 (TLR4) activation induces the secretion of TNF-α, IL-1β, IL-4, IL-6, IL-10, and IL-13 in rodent mast cells (McCurdy et al., 2003; Masuda et al., 2002; Supajatura et al., 2002; Sandig and Bulfone-Paus, 2012). The amount of cytokines produced by mast cells often exceeds that produced by the more traditional effector cells, such as macrophages and T cells (Leal-Berumen et al., 1994; Gupta et al., 1996). However, it is unclear whether METH regulates LPS-induced cytokine responses in mast cells.

Recent studies have demonstrated that D_3 receptors (D3Rs) are associated with METH addiction (Sokoloff et al., 2001, 2006; Le Foll et al., 2014; Chen et al., 2014). For example, a recent study showed that inhibition of the dopamine D3R attenuated the rewarding and incentive motivational effects of METH in rats (Chen et al., 2014). Our previous study showed that the D3R plays distinct roles in modulating METH-induced behavioural sensitization (Zhu et al., 2012). Therefore, D3Rs may be potential targets for the treatment of METH dependence (Higley et al., 2011; Tziortzi et al., 2011). These findings also prompted us to investigate whether D3R regulates the effect of METH on the immune response. Interestingly, a recent study indicated that the D3R was involved in METH-induced hyperthermia (Baladi et al., 2014). Previous studies showed that D3R altered the immune response of activated T cells and the migration and homing of naive $CD8^+$ T cells (Watanabe et al., 2006; Ilani et al., 2004). Additionally, the thymus expresses dopamine receptors, and METH treatment altered immunocompetent cell populations in the thymus of mice (In et al., 2005; Mignini et al., 2009). Furthermore, the lungs experience the highest METH uptake during consumption (Peerzada et al., 2013; Volkow et al., 2010), inducing significant lung injury (Wells et al., 2008). Moreover, the selective modulation of cytokines by dopamine is mediated by dopamine receptors expressed on immune cells lodged in the lungs (Matalka et al., 2011). Together, these studies indicate that METH may induce alterations in the thymic and lung immune responses, and that dopamine receptors may be involved in this immune response. However, it is unclear whether D3Rs are involved in METH-mediated immune responses induced by LPS stimulation.

In the current study, we investigated the effects of D3R on METH-mediated production of mast cell released cytokines in the lungs and thymi after LPS stimulation using D3R-deficient mice. We examined the mast cell released cytokines, including pro-inflammatory cytokines (IL-1β, IL-6, TNF-α), anti-inflammatory cytokines (IL-10), and Th2 response cytokines (IL-4 and IL-13), in the lungs and thymi of mice. To further understand the role of D3R in METH-induced mast cell alterations, we assessed the effects of a D3R agonist and antagonist on LPS-induced cytokine production following METH treatment in mouse bone marrow-derived mast cells (BMMCs). Our findings suggest that METH regulates mast cell released cytokines production in an LPS-induced mouse model *via* the D3R.

Methods

Animals

Dopamine D3R deficient ($D3R^{-/-}$) mice and wild-type (WT) mice were a kind gift from Xu et al. (Department of Anaesthesia and Critical Care, The University of Chicago). $D3R^{-/-}$ mice have been previously described (Zhu et al., 2012). $D3R^{-/-}$mice and control WT mice were bred under specific pathogen-free conditions in the same room of our animal facility. All experiments were performed in accordance with the guidelines of the Animal Ethics Committee of Xi'an Jiaotong University.

Drugs

METH hydrochloride was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, People's Republic of China). METH was dissolved in sterile 0.9% physiological saline, and 5 mg METH·HCl/kg body weight was injected i.m. *Escherichia coli* LPS (serotype O55:B5, Sigma-Aldrich, St. Louis, MO, USA) was dissolved in sterile saline, and 150 μg/kg body weight was injected i.m. Both METH and LPS were diluted such that the dose injection volume was 0.01 ml/g. Control mice received an equivalent volume of saline. 7-Hydroxy-(di-*n*-propylamino) tetralin (DPAT) hydrobromide (7-OH-DPAT) and NGB2904 hydrochloride were purchased from Sigma-Aldrich.

Experimental design and animal treatments

Six-week-old sex-matched $D3R^{-/-}$ and WT mice were randomized to 4 groups ($n = 6$), including normal saline (NS) + NS, METH + NS, NS + LPS and METH + LPS. The mice received four i.m. injections of METH or NS at 2 h intervals. Twenty-four hours after the first METH injection, the mice received i.m. injections of either LPS or saline. After 24 h, the mice were sacrificed by CO_2 asphyxiation, and tissues, including the lung and thymus, were collected for cytokine measurement and analysis.

Quantitative real-time PCR

Total RNA was extracted from the left lung and thymus and then purified using the TRIzol kit (Invitrogen, Carlsbad, CA, USA). Nucleic acid concentration and purity (A260/A280) were measured with a Thermo Scientific Microplate instrument (Thermo Fisher, Waltham, MA, USA). Residual genomic DNA was removed by incubation with RNase-free DNase. Reverse transcription was performed using a PrimeScript™RT reagent kit (Takara Bio Inc., Shiga, Japan) according to the manufacturer's instructions. The resulting cDNA was subjected to real-time PCR on a Stratagene Mx 3005p Real-Time PCR Detection System (Agilent Technologies, Santa Clara, CA, USA) using SYBR Green II as a double-strand DNA-specific binding dye to quantify the mRNA expression of TNF-α, IL-1β, IL-6, IL-10, IL-4 and IL-13 in lungs. The sequences of the forward (F) and reverse (R) primers are shown in Table 1. All primers were synthesized by Baosheng Biotech (Dalian, China). PCR amplification was performed in a total volume of 25 μl containing 1×10^{-5} mol each primer, 12.5 μl 2 × SYBR® Premix *Ex* Taq™II (Takara Bio Inc.), and nuclease-free PCR-grade water. The PCR protocol was: an initial denaturation at 95 °C for 30 s, 40 cycles at 95 °C for 5 s and 60 °C for 30 s, and 1 cycle at 95 °C for 1 min, 55 °C for 30 s, and 95 °C for 30 s. After completion of the reaction, specificity was verified by melting curve analysis. The results were expressed using the following equation: $2^{-\Delta\Delta Ct} = 2^{-(Ct\ target\ gene - Ct\ GAPDH)\ target\ sample - (Ct\ target\ gene - Ct\ GAPDH)\ control\ sample}$ (Livak and Schmittgen, 2001). The GAPDH gene was chosen as the