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Research Report

MK-801 and dextromethorphan block microglial activation and protect against methamphetamine-induced neurotoxicity

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Abstract

Methamphetamine causes long-term toxicity to dopamine nerve endings of the striatum. Evidence is emerging that microglia can contribute to the neuronal damage associated with disease, injury, or inflammation, but their role in methamphetamine-induced neurotoxicity has received relatively little attention. Lipopolysaccharide (LPS) and the neurotoxic HIV Tat protein, which cause dopamine neuronal toxicity after direct infusion into brain, cause activation of cultured mouse microglial cells as evidenced by increased expression of intracellular cyclooxygenase-2 and elevated secretion of tumor necrosis factor-α. MK-801, a non-competitive NMDA receptor antagonist that is known to protect against methamphetamine neurotoxicity, prevents microglial activation by LPS and HIV Tat. Dextromethorphan, an antitussive agent with NMDA receptor blocking properties, also prevents microglial activation. In vivo, MK-801 and dextromethorphan reduce methamphetamine-induced activation of microglia in striatum and they protect dopamine nerve endings against drug-induced nerve terminal damage. The present results indicate that the ability of MK-801 and dextromethorphan to protect against methamphetamine neurotoxicity is related to their common property as blockers of microglial activation.

Theme: Disorders of the Nervous System
Topic: Neurotoxicity

Keywords: Methamphetamine; Microglia; MK-801; Dextromethorphan; Dopamine; Neurotoxicity

1. Introduction

Abuse of amphetamines continues to increase at an alarming rate. In fact, the UN Office on Drugs and Crime reported that use of amphetamines, including methamphetamine (METH) and 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy), has eclipsed that of cocaine and heroin on a global scale. The problems associated with any drug of abuse are compounded in the case of the amphetamines because many members of this pharmacological class cause persistent damage to the central nervous system [55]. The mechanisms by which drugs like METH and MDMA damage monoaminergic nerve endings are not understood, but considerable evidence implicates oxidative stress as a contributing factor [12,31]. Neither the cellular source nor the identity of the reactive species that mediate amphetamine-induced neuronal damage is known.

Abbreviations: 5HT, serotonin; Cox-2, cyclooxygenase-2; DA, dopamine; DAB, 3,3′-diaminobenzidine; DXM, dextromethorphan; HRP, horseradish peroxidase; ILB_4, isolectin B_4; LPS, lipopolysaccharide; MDMA, 3,4-methylenedioxymethamphetamine; METH, methamphetamine; PBS, phosphate buffered saline; Tat_{72}, HIV Tat (residues 1–72); TNF-α, tumor necrosis factor-α

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Microglia are the primary antigen-presenting cells in the central nervous system. These immune-like cells [3] can be activated in response to injury, disease, or inflammation, leading to the secretion of a variety of factors such as proinflammatory cytokines, prostaglandins, and reactive oxygen/nitrogen species, each of which can cause neuronal damage [23,33]. In view of the fact that the neurotoxic effects of METH and MDMA are dependent on at least superoxide [10,11] and nitric oxide [26,27], both of which can be produced by microglia upon activation, it is somewhat surprising that microglial involvement in amphetamine-mediated neurotoxicity has received so little study. We have established recently that microglial activation is associated with the neurotoxic properties of METH, and not with other prominent pharmacological effects associated with this drug such as hyperthermia, stimulation of dopamine (DA) or serotonin (5HT) receptors, or inhibition of the DA and 5HT transporters [54]. Within the amphetamine class, those drugs that damage monoamine nerve endings (e.g., D-METH, MDMA, *p*-chloroamphetamine, and amphetamine) activate microglia whereas non-neurotoxic amphetamines (e.g., L-METH and DOI) do not [53].

Drugs that prevent microglial activation could bolster CNS defenses against the neuronal damage associated with stroke, injury, or neurodegenerative conditions. For example, minocycline is now known to have broad neuroprotective properties that have been attributed to its ability to block microglial activation [56]. We report presently that MK-801 and dextromethorphan block activation of cultured mouse microglial cells by LPS and the neurotoxic HIV Tat protein. In addition, these drugs protect against METH-induced neurotoxicity to DA nerve endings. Interruption of the microglial activation cascade in the early stages of METH intoxication may be part of the mechanism by which MK-801 and dextromethorphan limit drug-induced damage to DA nerve endings.

2. Materials and methods

2.1. Materials

(+) Methamphetamine hydrochloride, MK-801, dextromethorphan (DXM), DA, pentobarbital, horseradish peroxidase (HRP)-conjugated isolectin B_4 (ILB_4; from *Griffonia simplicifolia*), 3,3′-diaminobenzidine (DAB), paraformaldehyde, sodium orthovanadate, LPS (serotype 055:B5 from *E. coli*, cell culture tested), Triton X-100, and all buffers and HPLC reagents were purchased from Sigma-Aldrich (St. Louis, MO). A polyclonal antibody against cyclooxygenase-2 (Cox-2) was obtained from Cayman Chemical Co. (Ann Arbor, MI) and HRP-conjugated goat anti-rabbit IgG was purchased from Amersham Biosciences (Piscataway, NJ). CitriSolv and Permount were products of Fisher Scientific (Pittsburgh, PA). Western Lightning Chemiluminescence Reagent Plus was obtained from Perkin Elmer Life Sciences (Boston, MA) and Biomax MR film was from Kodak (Rochester, NY). Quantikine immunoassay kits for mouse tumor necrosis factor-α (TNF-α) were obtained from R&D Systems (Minneapolis, MN). The cDNA for full-length recombinant HIV-Tat was generously provided by Dr. Dinah Singer (NCI, NIH). A stop codon was inserted after amino acid residue 72 by PCR, and the resulting Tat_{72} construct was expressed as a His_6 fusion in EC BL21 cells and purified by NiNTA chromatography (Invitrogen). Tat_{72} represents the first exon of the full-length Tat construct and is sufficient for transactivation, cellular uptake, and neurotoxicity [38]. Purified Tat_{72} was treated with Detoxi-Gel Endotoxin Removing Gel (Pierce) before use in microglial cultures.

2.2. Microglial cell culture

BV-2 cells, a murine microglial cell line [6], were kindly provided by Dr. Paul M. Stemmer (Wayne State University EHS Center for Molecular and Cellular Toxicology with Human Applications). Cells were cultured at 37 °C in an atmosphere of 5% CO_2 in DMEM containing 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin (Gibco/BRL, Grand Island, NY). BV-2 cells were activated by treatment with LPS and HIV Tat_{72} because each has been associated with microglial-mediated damage to the DA neuronal system [1,19,20,29], and METH itself does not cause direct activation of microglial cells (data not shown). LPS (100 ng/ml) or Tat_{72} (200 ng/ml) were added to microglial cultures for 60 min at 37 °C after which the media was removed and replaced with fresh media lacking an activator. When MK-801 and DXM were tested for the ability to prevent LPS- or Tat_{72}-induced activation of microglial cells, they were added 60 min prior to the activators and remained in the media until cells were harvested for analysis after 24 h. Controls were treated with MK-801 or DXM in the absence of LPS or Tat_{72}. Activation of BV-2 cells was determined by analysis of Cox-2 protein expression and secretion of TNF-α into the medium, both of which are robust markers of microglial activation [5,23]. After removal of medium, cells were washed and homogenized in 1% SDS, 10 mM Tris pH 7.4, 1 mM sodium orthovanadate at 95 °C. Insoluble material was sedimented by centrifugation and solubilized proteins (equal amounts per lane across all treatment conditions) were resolved by SDS-PAGE and transferred to nitrocellulose paper. Cox-2 immunoreactivity was visualized with HRP-conjugated goat anti-rabbit IgG using enhanced chemiluminescence. Cox-2 immunoreactive bands were digitized and relative pixel densities were compared to determine changes in Cox-2 expression. TNF-α was quantified in culture medium by immunoassay according to manufacturer's instructions.