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SN79, a sigma receptor ligand, blocks methamphetamine-induced microglial activation and cytokine upregulation

Matthew J. Robson^a, Ryan C. Turner^a, Zachary J. Naser^a, Christopher R. McCurdy^b, Jason D. Huber^a, and Rae R. Matsumoto^a

^aDepartment of Basic Pharmaceutical Sciences, School of Pharmacy, West Virginia University 1 Medical Center Dr., West Virginia University Health Sciences Center, Morgantown, WV 26506

^bDepartment of Medicinal Chemistry and Department of Pharmacology, School of Pharmacy, University of Mississippi, P.O. Box 1848, University, MS 38677-1848

Abstract

Methamphetamine (METH) abuse is associated with several negative side effects including neurotoxicity in specific brain regions such as the striatum. The precise molecular mechanisms by which METH usage results in neurotoxicity remain to be fully elucidated, with recent evidence implicating the importance of microglial activation and neuroinflammation in damaged brain regions. METH interacts with sigma receptors which are found in glial cells in addition to neurons. Moreover, sigma receptor antagonists have been shown to block METH-induced neurotoxicity in rodents although the cellular mechanisms underlying their neuroprotection remain unknown. The purpose of the current study was to determine if the prototypic sigma receptor antagonist, SN79, mitigates METH-induced microglial activation and associated increases in cytokine expression in a rodent model of METH-induced neurotoxicity. METH increased striatal mRNA and protein levels of cluster of differentiation 68 (CD68), indicative of microglial activation. METH also increased ionized calcium binding adapter molecule 1 (IBA-1) protein expression, further confirming the activation of microglia. Along with microglial activation, METH increased striatal mRNA expression levels of IL-6 family pro-inflammatory cytokines, leukemia inhibitory factor (*Lif*), oncostatin m (*osm*), and interleukin-6 (*il-6*). Pretreatment with SN79 reduced METH-induced increases in CD68 and IBA-1 expression, demonstrating its ability to prevent microglial activation. SN79 also attenuated METH-induced mRNA increases in IL-6 pro-inflammatory cytokine family members. The ability of a sigma receptor antagonist to block METH-induced microglial activation and cytokine production provides a novel mechanism through which the neurotoxic effects of METH may be mitigated.

Keywords

methamphetamine; sigma receptor; microglial activation; cytokine; neurotoxicity

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Corresponding author: Rae R. Matsumoto, West Virginia University, School of Pharmacy, P.O. Box 9500, Morgantown, WV 26506. Phone: 1-304-293-1450; FAX: 1-304-293-2576. rmatsumoto@hsc.wvu.edu.

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Introduction

Methamphetamine (METH) is an addictive psychostimulant that is currently the second most abused illicit substance in the world behind only Cannabis (Romanelli and Smith, 2006). METH exerts many of its behavioral and physiologic effects by modulating monoaminergic systems within the central nervous system (CNS). METH abuse has several associated side effects including mood disturbances, anxiety, severe dental problems and notably, neurotoxicity (Romanelli and Smith, 2006). Chronic METH usage has been shown in humans to result in dopaminergic damage in the striatum, an effect correlated to relapse rates during clinical addiction treatment (Volkow et al., 2001a; Volkow et al., 2001b; Wang et al., 2012). Recently, chronic abusers of METH were also found to have a greater risk of developing Parkinson's disease later in life, which is believed to stem from the neurotoxic consequences of the drug (Callaghan et al., 2010; Callaghan et al., 2012).

The precise mechanisms by which METH elicits neurotoxic effects are still being elucidated. There are several contributing factors that have been implicated in these effects, such as increased reactive oxygen and nitrogen species (ROS/RNS) generation, dopamine quinone formation, caspase and cell death signaling cascade activation, endoplasmic reticulum (ER) stress induction, and glutamatergic excitotoxicity (Cadet and Krasnova, 2009; Krasnova and Cadet, 2009). Additionally, microglial activation has been implicated in the neurotoxic effects of METH (Escubedo et al., 1998; Kelly et al., 2012; Kuhn et al., 2006; Thomas et al., 2004a; Yue et al., 2012).

Microglia are the resident macrophages of the CNS that function in maintaining homeostasis by sensing deviations from the normal brain environment. Upon activation by perturbations of their surrounding environment, microglia can undergo transformation to different response phenotypes, similar to peripheral macrophages (Perry et al., 2010; Saijo and Glass, 2011). These have classically been categorized as M1- and M2-type macrophage/microglia, with M1 microglia being associated with inflammation and degeneration and M2 being associated with regeneration or anti-inflammatory processes (Czeh et al., 2011; Mosser and Edwards, 2008). The classical activation of microglia (M1) is associated with an upregulation of a variety of cell surface proteins, release of pro-inflammatory cytokines, generation of ROS/RNS, and subsequent neuronal damage (Czeh et al., 2011; Mosser and Edwards, 2008; Perry et al., 2010), all of which have been shown to be increased in response to neurotoxic regimens of METH (Escubedo et al., 1998; Kuhn et al., 2006; O'Callaghan et al., 2008; Thomas et al., 2004a; Thomas et al., 2004b).

The activation of microglia by METH has been demonstrated in both human studies and preclinical rodent models (Sekine et al., 2008; Thomas et al., 2004a; Thomas et al., 2004b). Microglial activation by METH occurs in regions of the brain affected by the neurotoxic actions of the drug and these effects have been shown to be persistent and long lasting even after extended abstinence from the drug (Sekine et al., 2008). The ability of microglia to produce cytokines and reactive species that can compromise synaptic transmission and neuronal function make them logical contributors to METH neurotoxicity and intriguing targets for drug development.

Among the pro-inflammatory cytokines, members of the interleukin-6 (IL-6) family are of particular relevance in the context of METH neurotoxicity. It is believed that the release of cytokines (including IL-6-type cytokines) by activated microglia is relevant to the effects of several neurodegenerative disorders, representing mechanistic overlap between these diseases and the neurotoxic actions of METH (Cadet and Krasnova, 2009; Smith et al., 2012). The upregulation of IL-6 and other members of this cytokine family have been shown in brain regions affected by the neurotoxic actions of METH (Goncalves et al., 2008; Kelly

et al., 2012). Moreover, it has previously been shown that mice lacking IL-6 are protected against the neurotoxic actions of METH (Ladenheim et al., 2000), supporting a role of IL-6 in the neurotoxic actions of the drug. Furthermore, it is believed that molecular signaling cascades activated by IL-6-type cytokine signaling are involved in glial cell activation by METH (Hebert and O'Callaghan, 2000).

Presently, there are no FDA approved medications aimed at treating any of the negative side effects of METH abuse, including neurotoxicity. Many potentially promising preclinical treatments have failed to provide clinically effective pharmacotherapies, including those targeting monoaminergic systems. Sigma receptors have recently emerged as a potential target for the production of novel therapeutics aimed at treating many of the negative effects associated with METH usage, including neurotoxicity (Robson et al., 2012; Rodvelt and Miller, 2010). There are currently two known subtypes of sigma receptors, denoted sigma-1 and sigma-2, and sigma receptor antagonists have been shown to mitigate METH-induced behavioral effects and hyperthermia in rodents (Matsumoto et al., 2008; Nguyen et al., 2005). Additionally, sigma receptor antagonists block METH-induced reductions in striatal dopamine and serotonin and their respective transporters in preclinical models of METH-induced neurotoxicity (Kaushal et al., 2012b; Seminerio et al., 2011). The ability of sigma receptor antagonists to mitigate the effects of METH on neuronal cells is well documented; however, it is currently unclear if sigma receptor antagonists also block METH-induced microglial activation.

Sigma receptors are found in microglial cells and sigma receptor ligands have been shown to modulate microglial activation *in vitro* and *in vivo* (Cuevas et al., 2011; Hall et al., 2009). Sigma receptor ligands have been shown to modulate several aspects of microglial activation including migration and cytokine release in response to various activators such as adenosine triphosphate (ATP) and lipopolysaccharide (LPS) (Cuevas et al., 2011; Hall et al., 2009). The sigma receptor ligand SR 31747 has also been reported to block peripheral increases in IL-6 expression in response to peripheral LPS and Staphylococcal enterotoxin B administration, although data from the CNS is currently lacking (Bourrie et al., 1996; Derocq et al., 1995). These studies provide evidence that sigma receptor ligands are capable of modulating the functionality of immune cells, including those present within the CNS. The purpose of the current study was therefore to determine if the putative sigma receptor antagonist and drug development candidate, 6-acetyl-3-(4-(4-(4-fluorophenyl)piperazin-1-yl)butyl)benzo[d]oxazol-2(3H)-one (SN79), mitigates microglial activation and cytokine upregulation elicited by METH in the striatum in a preclinical model of METH-induced neurotoxicity. This information will be significant for identifying therapeutically relevant targets for the actions of sigma ligands such as SN79, and sets the stage for future detailed mechanistic studies.

Materials and methods

Drugs and chemicals

(+)-Methamphetamine hydrochloride was obtained from Sigma Aldrich (St. Louis, MO). SN79 was synthesized as previously described (Kaushal et al., 2011b) and provided by Dr. Christopher R. McCurdy (University of Mississippi, University, MS). All administered drugs were dissolved in sterile saline solution (0.1 mL/10 g body weight) (Teknova, Fisher Scientific, Pittsburgh, PA). All other chemicals were obtained from Sigma Aldrich (St. Louis, MO) unless otherwise specified.

Animals

Male, Swiss Webster mice (24-28 g; Harlan, Indianapolis, IN) were utilized for all experiments. Mice were housed in groups of five, on a 12:12 h light/dark cycle with food and water *ad libitum*. Mice were randomly assigned to their respective treatment groups for all experiments. Experiments were performed as approved by the Animal Care and Use Committee at West Virginia University.

Repeated dosing paradigm

Mice were randomly distributed for each experiment into one of four treatment groups: 1) Saline/Saline (0.1 mL/10 g body weight), 2) Saline/METH 5 mg/kg, 3) SN79 3 mg/kg/Saline, or 4) SN79 3 mg/kg/METH 5 mg/kg. The first compound listed in each pair (Saline or SN79) was administered as a pretreatment 15 min prior to the second compound in each treatment group (Saline or METH). Each animal underwent four pretreatments/treatments 2 h apart as previously described (Kaushal et al., 2012b). All injections were administered intraperitoneally.

Core body temperature was measured 1 h after each treatment using a probe (Thermalert TH-S Monitor, Physitemp Instruments Inc., Clifton, NJ) inserted approximately 2.5 cm past the rectum into the colon. Body temperature was recorded after a stable temperature had been reached approximately 10 s post-probe insertion.

The METH dose (5 mg/kg $\times$ 4) was selected based upon previous dose response experiments assessing dopaminergic neurotoxicity in this specific model, where it has consistently been shown to result in significant dopaminergic deficits in the striatum (Matsumoto et al., 2008; Seminerio et al., 2012). Similarly, the SN79 dose (3 mg/kg $\times$ 4) was selected due to previously reported dose response experiments assessing the ability of this compound to mitigate the striatal dopaminergic deficits elicited by METH (Kaushal et al., 2012b).

At various time points post-treatment (as measured from the last injection) bilateral striatum samples were collected, flash frozen in liquid nitrogen and stored at -80°C for later use. Samples for protein level analysis were collected 72 h post-treatment and were collected after transcardial perfusion with phosphate buffered saline (PBS) followed by 4% paraformaldehyde. Time points were selected based upon time course experiments contained within this report (microglial markers and cytokine expression levels) and previous data reporting the timeline of METH-induced dopaminergic neurotoxicity and microglial activation in rodent models (Escubedo et al., 2005; Escubedo et al., 1998; Kita et al., 2000; Thomas et al., 2004b).

Quantitative real-time PCR

Total RNA was extracted from flash frozen striatum samples using Trizol reagent (Invitrogen, Grand Island, NY) according to manufacturer's instructions. The total RNA concentration for each sample was quantified by spectral absorption, and the purity of the sample checked to confirm that the 260/280 ratio was in the range of 1.8-2.0. Samples of cDNA were prepared by reverse transcription using a high capacity reverse transcription kit (Applied Biosystems, Foster City, CA). Each sample reaction included MultiScribeTM Reverse Transcriptase and random primers, with thermal cycler conditions set as follows: step 1 at 25°C for 10 min, step 2 at 37°C for 120 min, step 3 at 85°C for 5 s, and step 4 at 4°C for 10 min.

For the PCR amplification, TaqMan[®] Universal PCR Master Mix and the following probes were obtained from Applied Biosystems (Foster City, CA): 18s (Hs99999901_s1) for use as

an endogenous control gene, IL6 (Mm00446190_m1), OSM (Mm0119366_m1), CD68 (Mm03047343_m1) and Lif (Mm00434762_g1). The reaction mixture was prepared according to the manufacturer's instructions, with the following thermal cycling conditions: initial holding at 50° C for 2 min which is required for optimal AmpErase® UNG activity, followed by a first denaturing step at 95° C for 10 min, then 45 cycles at 95° C for 15 s, and at 60° C for 1 min. The data from the real-time PCR measurements were calculated using the $\Delta\Delta C_t$ method. The threshold value was set at 0.2 and the threshold cycle (C_t value) of each gene was normalized to 18s rRNA.

Immunohistochemistry

At 72 h post-treatment (after the last injection), animals were anesthetized and immediately perfused transcardially with PBS followed by 4% paraformaldehyde. The entire brain was removed and immediately placed in 4% paraformaldehyde for 24 h. Following fixation, the brains were processed using a Tissue-Tek VIP 5 automatic tissue processor (Sakura Finetek, Torrance, CA) and then embedded in paraffin with a Tissue-Tek TEC 5 embedding console system (Sakura Finetek). Embedded tissues were sliced in 6 μ m sections using a Leica RM2235 microtome (Leica Microsystems, Buffalo Grove, IL), and slices mounted on glass slides for staining. All slides were heat-fixed and deparaffinized via a series of xylene and alcohol washes prior to immunohistochemical procedures. Classical (M1) and alternative (M2) labeling of microglia was conducted as previously described (Niino et al., 2010; Zhang et al., 2012). Briefly, tissues were incubated first in mouse anti-rat CD68 antibody (AbD Serotec, Kidlington, UK) at a dilution of 1:100 in 4% horse serum in Dulbecco's phosphate buffered saline (DPBS) overnight. Sections were washed 3 $\times$ in DPBS and then incubated in a biotinylated anti-rabbit IgG secondary antibody (Vector Laboratories, Burlingame, CA) diluted at 1:10,000 in 4% horse serum in DPBS for 4 h. Following incubation with a secondary antibody, alkaline phosphatase (Life Technology, Carlsbad, CA) diluted at 1:100 in Tris-bovine serum albumin (BSA) was applied for 1 h. Following the incubation with alkaline phosphatase, the tissues were rinsed 3 $\times$ in DPBS and then Fast Blue BB salt (Santa Cruz Biotechnology, Santa Cruz, CA) for 5 min. Tissues were again rinsed 3 $\times$ in DPBS prior to incubation in mouse anti-rat CD163 (AbD serotec, Kidlington, UK) at a dilution of 1:100 in 4% horse serum in DPBS overnight. Following incubation with the primary antibody, the tissues were washed 3 $\times$ in DPBS and then incubated in a biotinylated anti-rabbit IgG secondary antibody (Vector Laboratories, Burlingame, CA) diluted at 1:10,000 in 4% horse serum in DPBS for 4 h. Following incubation with the secondary antibody, the tissues were then incubated in avidin D-horseradish peroxidase (HRP) (Vector Laboratories, Burlingame, CA) diluted at 1:1,000 in DPBS for 1 h. The chromagen solution, diaminobenzidine (DAB) (Vector Laboratories, Burlingame, CA), was applied for 5 min per manufacturer's instructions. The tissues underwent a final wash in xylene and were mounted using an antifade agent and cover slipped. The slides were sealed with acrylic and stored in the dark in a laboratory refrigerator. Staining for IBA-1, another marker of microglial activation, followed a similar approach but utilized anti-IBA-1 polyclonal antibody raised in rabbit (Wako, Richmond, VA). Sections were developed using Nova Red (Vector Laboratories, Burlingame, CA).

Statistical analysis

Statistical analyses were conducted using analysis of variance (ANOVA). For significant effects, pairwise comparisons followed using post hoc Bonferroni's or Tukey's multiple comparisons tests. $p < 0.05$ was considered statistically significant.

Results

Body temperature modulation

Dosing regimens of METH previously found to be neurotoxic (Matsumoto et al., 2008; Seminerio et al., 2012) significantly increased core body temperature in mice, as compared to saline-treated controls. Two-way repeated measures ANOVA revealed significant differences between treatment groups and time, as well as their interaction ($p < 0.0001$, $p < 0.0001$ and $p < 0.0001$, respectively). Bonferroni's post hoc analyses revealed that METH (5 mg/kg $\times$ 4) treatment significantly increased body temperature as compared to saline at time points 2, 3 and 4 ($t = 5.16$, $p < 0.001$, $t = 9.45$, $p < 0.001$ and $t = 10.39$, $p < 0.001$, respectively) (Fig. 1). SN79 pretreatment was also found to significantly attenuate METH-induced changes in body temperature at all four time points (1: $t = 7.28$, $p < 0.001$; 2: $t = 8.50$, $p < 0.001$; 3: $t = 8.93$, $p < 0.001$ and 4: $t = 10.38$, $p < 0.001$), while having no effects on its own as compared to saline-treated animals (n.s. for time points 1, 2, 3 and 4) (Fig. 1).

Markers of microglial activation

CD68 expression is a commonly used marker of microglial activation due to CNS insult (Komohara et al., 2011; Li et al., 2009; Nagai et al., 2005). Two-way ANOVA revealed significant differences in striatal *cd68* mRNA expression between treatment groups, time, and their interaction ($p < 0.0001$ for treatment, time, and their interaction). Post hoc Bonferroni's analyses revealed that METH (5 mg/kg $\times$ 4) caused a significant increase in striatal *cd68* expression at 3, 6, 12 and 24 h post-treatment as compared to saline alone ($t = 3.15$, $p < 0.05$, $t = 4.37$, $p < 0.001$, $t = 8.58$, $p < 0.001$ and $t = 13.06$, $p < 0.001$, respectively) (Fig. 2).

At 24 h post-treatment, a time point when the highest METH effects were observed, significant differences between treatment groups in *cd68* mRNA expression were found (one-way ANOVA; $F[3,39] = 37.84$, $p < 0.0001$). Post hoc analysis confirmed that similar to the time course study, METH (5 mg/kg $\times$ 4) treatment resulted in a significant increase in striatal *cd68* expression as compared to saline alone ($q = 13.63$, $p < 0.001$). This METH-induced change in *cd68* expression was significantly attenuated by SN79 pretreatment (3 mg/kg $\times$ 4) ($q = 9.11$, $p < 0.001$), though the blockade was incomplete since SN79 pretreatment prior to METH still resulted in an elevated expression of *cd68* as compared to saline-treated controls ($q = 4.51$, $p < 0.05$). In the absence of METH, SN79 treatment alone resulted in no significant changes in striatal *cd68* mRNA expression (n.s.) (Fig. 3A).

To further confirm that SN79 mitigated processes related to METH-induced microglial activation, protein expression levels were determined for both CD68 and the commonly used microglial marker IBA-1 using immunohistochemistry at a later time point of 72 h post-treatment. One-way ANOVA revealed significant differences between treatment groups in CD68 protein expression at 72 h ($F[3,19] = 24.17$, $p < 0.0001$). Tukey's post hoc analysis revealed that METH treatment significantly increased striatal CD68 protein expression as compared to saline ($q = 9.70$, $p < 0.001$). In accordance with the mRNA expression studies, SN79 pretreatment attenuated the striatal CD68 protein increases elicited by METH ($q = 9.67$, $p < 0.001$), while having no effect on its own (n.s.) (Fig. 3B).

Similar to the pattern of effects observed for CD68 protein, SN79 pretreatment also blocked METH-induced increases in striatal IBA-1 protein expression, providing confirmation of a reduction in another marker of microglial activation. One-way ANOVA revealed significant differences between groups in IBA-1 expression ($F[3,19] = 150.00$, $p < 0.0001$). Post hoc analyses showed that METH treatment increased IBA-1 expression as compared to saline-treated controls at 72 h ($q = 26.35$, $p < 0.001$), which was blocked by SN79 pretreatment ($q = 24.40$, $p < 0.001$). Interestingly, SN79 alone resulted in a slight, yet statistically

significant, paradoxical increase in IBA-1 expression compared to saline-treated controls ($q = 4.41$, $p < 0.05$) (Fig. 4).

Immunohistochemical studies to determine the type of microglia (M1 or M2) that was being activated by METH within the striatum revealed a strong, preferential effect on M1 microglia. While METH treatment resulted in a significant increase in M1-type microglia (denoted by solely expressing CD68; as shown in Fig. 3B), it did not increase nor decrease levels of M2-type microglia in the striatum (denoted by co-expression of CD68 and CD163; one-way ANOVA, n.s.). Additionally, SN79 treatment had no effect on M2-type microglia in the striatum, either alone or in the presence of METH (n.s.) (Fig. 5).

IL-6 family inflammatory cytokine mRNA expression

Along with the activation of pro-inflammatory M1 microglia, METH increased the mRNA expression of *il-6*, *osm* and *lif*, three IL-6 family cytokines within the striatum in a time-dependent manner. Two-way ANOVA revealed differences in *il-6* expression between treatment groups ($p < 0.0001$). Post hoc analyses revealed that METH significantly increased *il-6* mRNA expression at 1.5, 3, 6, 12 and 24 h ($t = 3.69$, $p < 0.01$, $t = 5.87$, $p < 0.001$, $t = 4.94$, $p < 0.001$, $t = 6.25$, $p < 0.001$ and $t = 3.09$, $p < 0.05$, respectively) (Fig. 6A).

Two-way ANOVA also revealed significant differences between treatment groups, time, and their interaction in striatal *osm* mRNA expression ($p < 0.0001$, $p < 0.05$ and $p < 0.05$, respectively). Post hoc tests revealed that METH significantly increased *osm* expression as compared to saline treatment at 1.5, 3, 6 and 12 h post-treatment ($t = 5.68$, $p < 0.001$, $t = 6.41$, $p < 0.001$, $t = 5.86$, $p < 0.001$ and $t = 5.4$, $p < 0.001$, respectively) (Fig. 6B).

A significant difference was also found in striatal *lif* expression as a result of METH treatment, with two-way ANOVA showing a significant difference between treatment groups, time, and their interaction ($p < 0.0001$, $p < 0.01$ and $p < 0.01$, respectively). Post hoc analyses confirmed that METH treatment significantly increased *lif* expression in the striatum at 1.5, 3, 6, and 12 h post treatment as compared to saline ($t = 6.02$, $p < 0.001$, $t = 7.36$, $p < 0.001$, $t = 4.73$, $p < 0.001$ and $t = 3.11$, $p < 0.05$, respectively) (Fig. 6C).

At 6 h post-treatment, a time point at which all three IL-6 family cytokines studied were significantly up-regulated by METH in the time course studies described above, pretreatment with SN79 blocked these effects. One-way ANOVA revealed significant differences in striatal *il-6* mRNA expression between the treatment groups at 6 h ($F[3,38] = 21.16$, $p < 0.0001$). Similar to the time course experiments described above, METH treatment increased striatal *il-6* expression as compared to saline-treated controls ($q = 10.00$, $p < 0.001$), an effect mitigated by SN79 pretreatment ($q = 6.38$, $p < 0.001$). SN79 treatment alone was also found to have no effect on striatal *il-6* mRNA expression (n.s.) (Fig. 7A).

Pretreatment with SN79 also mitigated METH-induced increases in *osm* expression in the striatum. One-way ANOVA showed significant differences in *osm* expression between treatment groups ($F[3,38] = 19.48$, $p < 0.0001$). Tukey's post hoc analysis revealed that METH significantly increased *osm* mRNA expression as compared to saline controls ($q = 9.45$, $p < 0.001$). SN79 treatment attenuated METH-induced increases in *osm* expression ($q = 5.57$, $p < 0.001$), while having no effects on its own compared to saline-treated controls (n.s.) (Fig. 7B).

Additionally, SN79 attenuated METH-induced increases in *lif* expression in the striatum. One-way ANOVA showed a difference between the treatment groups in striatal *lif* expression ($F[3,38] = 31.89$, $p < 0.0001$), and post hoc analysis revealed that METH caused a significant increase in *lif* as compared to saline controls ($q = 11.99$, $p < 0.001$). SN79

blocked METH-induced increases in *lilf* mRNA expression ($q = 9.10$, $p < 0.001$), while having no effects on its own (n.s.) (Fig. 7C).

Discussion

Data from the current study provides evidence that sigma receptor antagonists, such as SN79, block METH-induced microglial activation. SN79 blocked METH-induced increases in mRNA and protein of the microglial markers CD68 and IBA-1. Additionally, SN79 treatment blocked METH-induced increases in the expression of pro-inflammatory IL-6 family cytokines in the striatum. The ability of SN79 to block METH-induced microglial activation and increases in the expression of pro-inflammatory cytokines provides evidence that this may be a mechanism by which sigma receptor antagonists mitigate the neurotoxic effects of METH.

The characterization of microglial activation as a result of METH exposure in preclinical rodent models has provided evidence that microglial activation is associated with METH-induced neurotoxicity (Kelly et al., 2012; LaVoie et al., 2004; Thomas and Kuhn, 2005; Thomas et al., 2004b). METH activates microglia in a dose- and time-dependent manner coinciding with reductions in striatal dopamine levels elicited by exposure to the drug (Thomas et al., 2004b). Amphetamine-type drugs that do not cause neurotoxicity such as L-methamphetamine and fenfluramine, fail to activate microglia, providing further evidence that microglial activation is associated with neurotoxicity (Thomas et al., 2004a). Additionally, environmental manipulations that block METH-induced dopaminergic neurotoxicity such as reductions in ambient temperature also block METH-induced microglia activation (Thomas et al., 2004b). This same study, however, also ruled out hyperthermia as a causative factor in microglia activation as hyperthermia elicited by simple ambient temperature increases or pharmacologic treatments other than METH were unable to cause microglial activation (Thomas et al., 2004b). These results thus imply that hyperthermia plays a significant contributory, but not causative, role in microglia activation by METH, and compounds such as SN79 may convey beneficial effects at least in part through the blockade of METH-induced hyperthermia.

The current study fails to determine the specific relationship between SN79's ability to mitigate METH-induced hyperthermia and the ability to block METH-induced microglial activation; however it is known that SN79 has neuroprotective effects against METH in the presence of elevated temperatures *in vitro* (Kaushal et al., 2012a; Kaushal et al., 2011a). Sigma receptors themselves and ligands targeting sigma receptors, including SN79, are known to modulate molecular signaling related to cellular toxicity, including those hypothesized to be involved in METH neurotoxicity (Hayashi and Su, 2007; Kaushal et al., 2012a; Kaushal et al., 2011a; Kaushal et al., 2012b; Mori et al., 2012; Smith et al., 2010). Therefore, it is plausible that the combination of alterations in cellular signaling related to METH-induced neurotoxicity and the mitigation of METH-induced increases in core body temperature contribute to the ability of SN79 to convey protective effects against METH *in vivo*. Additionally, the ability of SN79 to mitigate METH-induced hyperthermia may be clinically relevant, as METH-induced hyperthermia is believed to be a contributing factor in lethality as a result of METH overdose (Bowyer et al., 1994; Cruickshank and Dyer, 2009; Romanelli and Smith, 2006).

Increases in pro-inflammatory cytokines in the striatum following a neurotoxic regimen of METH have been shown previously (Kelly et al., 2012; Kuhn et al., 2006). Recently, increases in striatal expression levels of IL-6 family pro-inflammatory cytokines such as OSM, LIF and IL-6 by METH exposure were reported (Kelly et al., 2012). Further support of a role for IL-6 and its family members in the neurotoxic effects of METH are studies

showing a reduction in dopaminergic and serotonergic neurotoxicity by METH in IL-6 knockout mice (Ladenheim et al., 2000). These effects were found independently of any alterations in METH-induced hyperthermia between knockout animals and wild type controls, indicative of a specific effect of IL-6 reduction on neurotoxicity (Ladenheim et al., 2000). The activation of microglia with a concomitant increase in pro-inflammatory cytokines such as IL-6 is believed to result in neuronal toxicity through excitotoxicity, apoptosis, immune activation, and generation of ROS/RNS, all of which are hypothesized to contribute to the neurotoxic effects of METH (Cadet et al., 2003; Cadet and Krasnova, 2009; Smith et al., 2012). Additionally, the extent of dopaminergic nerve terminal damage has recently been found to be linked to microglial activation, further inferring that microglial activation and neuroinflammation may play a prominent role in the ability of METH to elicit neurotoxic responses (Kelly et al., 2012; Thomas and Kuhn, 2005).

The ability of sigma receptor antagonists such as SN79 to block microglial activation and increases in IL-6 family cytokine mRNA elicited by METH may be one mechanism by which they are able to mitigate the neurotoxic effects of METH. Sigma receptors themselves are known to be expressed in microglia and ligands targeting sigma receptors have been shown to modulate microglia function and activation in *in vitro* and *in vivo* models (Ajmo et al., 2006; Hall et al., 2009; Ruscher et al., 2012). The precise mechanism by which this occurs is currently unknown; however, sigma receptor modulation alters intracellular Ca^{2+} levels that have been associated with microglial activation and migration *in vitro* (Cuevas et al., 2011; Hall et al., 2009). This effect does not appear to be subtype specific, as ligands targeting sigma-1 and sigma-2 receptors independently have been shown to modulate microglial responses to a variety of microglial activators such as ATP, uridine triphosphate (UTP), LPS and monocyte chemoattractant protein-1 (Cuevas et al., 2011; Hall et al., 2009; Yao et al., 2010). The ability of sigma receptor ligands to alter intracellular Ca^{2+} levels within microglia, thereby altering activation and migration, may result in the attenuation of the production of pro-inflammatory cytokines in activated microglia and the subsequent neurotoxicity associated with METH treatment (Cuevas et al., 2011).

It should be noted that although ligands targeting sigma receptors have been shown to modulate microglial responses *in vitro* and *in vivo*, the specific linking of these effects to sigma receptors through the use of genetic knockout/knockdown studies has yet to be conducted. Compounding this problem is the current lack of molecular and selective pharmacologic tools for the study of the roles of sigma-2 receptors in various models. Future studies aimed at delineating the subtype(s) responsible for effects seen here, as well as specifically determining sigma receptor involvement through the use of genetic manipulations are warranted.

Interestingly, METH-induced microglial activation has also been linked to behavioral effects associated with usage of the drug in addition to neurotoxicity. Minocycline is an anti-inflammatory agent known to affect microglia that has been shown in rodent models to block METH-induced increases in locomotor activity, development of behavioral sensitization, impairments in recognition memory and rewarding effects of the drug as assessed by a conditioned place preference paradigm (Hashimoto et al., 2007; Mizoguchi et al., 2008; Zhang et al., 2006). A recent case report has also provided clinical evidence that minocycline treatment was linked to a reduction in METH-induced psychosis in a patient experiencing hallucinations associated with chronic METH usage (Tanibuchi et al., 2010). These reports provide evidence of a link between microglial activation and the behavioral and neurotoxic effects of METH. Notably, sigma receptor antagonists have been shown in rodent models to block METH-induced increases in locomotor activity, development and expression of behavioral sensitization and impairments in recognition memory, in addition to blocking hyperthermia and neurotoxicity associated with the drug (Kaushal et al., 2012b;

Kaushal et al., 2011c; Seminerio et al., 2012; Seminerio et al., 2011). The current report however, is the first showing that sigma receptor antagonists are able to mitigate METH-induced microglial activation. It remains to be seen if the ability of sigma receptor antagonists to mitigate the aforementioned METH-induced behaviors is associated with modulation of microglial activity.

It has recently been hypothesized that compounds targeting glial cells may be effective clinical treatments for the abuse of psychostimulants, including METH (Cooper et al., 2012). Given the current lack of treatments aimed at reducing METH usage or the negative effects associated with chronic METH usage, the further study of compounds that modulate glial cell function is certainly warranted. Compounds such as SN79 may be promising candidates to treat the negative complications of psychostimulant abuse by acting through a variety of mechanisms including microglial activation. Moreover, the ability of sigma ligands to reduce the hyperthermic effect of METH is therapeutically desirable and of clinical relevance since hyperthermia has been implicated as a contributing factor in other undesirable effects of METH including neurotoxicity. SN79 has previously been shown to display pharmacokinetic parameters amenable to clinical usage, and is orally bioavailable (Kaushal et al., 2011b), making it a potentially viable compound for further drug development. Future studies aimed at further delineating the mechanisms by which sigma receptor antagonists mitigate the neurotoxic actions of METH and further exploring drug development of these compounds for clinical usage are warranted.

Conclusion

METH causes neurotoxicity in the striatum, an effect associated with microglial activation and neuroinflammation. Sigma receptor antagonists have been shown to block a variety of METH-induced behaviors and side effects such as neurotoxicity. In the current study, the prototypic sigma receptor antagonist, SN79, was found to block METH-induced microglial activation and increases in expression of IL-6 family pro-inflammatory cytokines in the striatum. The blockade of striatal microglial activation and cytokine generation indicates one potential mechanism by which sigma receptor antagonists mitigate the neurotoxic effects of METH. Furthermore, properties of SN79 make it an amenable drug development candidate aimed at treating the negative side effects of METH usage.

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References

- Ajmo CT Jr, Vernon DO, Collier L, Pennypacker KR, Cuevas J. Sigma receptor activation reduces infarct size at 24 hours after permanent middle cerebral artery occlusion in rats. *Curr Neurovasc Res.* 2006; 3:89–98. [PubMed: 16719792]
- Bourrie B, Benoit JM, Derocq JM, Esclangon M, Thomas C, Le Fur G, Casellas P. Staphylococcal enterotoxin B-induced cytokine production in mice. *Immunology.* 1996; 31747A; 88:389–393. [PubMed: 8774355]
- Bowyer JF, Davies DL, Schmued L, Broening HW, Newport GD, Slikker W Jr, Holson RR. Further studies of the role of hyperthermia in methamphetamine neurotoxicity. *J Pharmacol Exp Ther.* 1994; 268:1571–1580. [PubMed: 8138969]
- Cadet JL, Jayanthi S, Deng X. Speed kills: cellular and molecular bases of methamphetamine-induced nerve terminal degeneration and neuronal apoptosis. *FASEB J.* 2003; 17:1775–1788. [PubMed: 14519657]

- Cadet JL, Krasnova IN. Molecular bases of methamphetamine-induced neurodegeneration. *Int Rev Neurobiol.* 2009; 88:101–119. [PubMed: 19897076]
- Callaghan RC, Cunningham JK, Sajeev G, Kish SJ. Incidence of Parkinson's disease among hospital patients with methamphetamine-use disorders. *Mov Disord.* 2010; 25:2333–2339. [PubMed: 20737543]
- Callaghan RC, Cunningham JK, Sykes J, Kish SJ. Increased risk of Parkinson's disease in individuals hospitalized with conditions related to the use of methamphetamine or other amphetamine-type drugs. *Drug Alcohol Depend.* 2012; 120:35–40. [PubMed: 21794992]
- Cooper ZD, Jones JD, Comer SD. Glial modulators: a novel pharmacological approach to altering the behavioral effects of abused substances. *Expert Opin Investig Drugs.* 2012; 21:169–178.
- Cruickshank CC, Dyer KR. A review of the clinical pharmacology of methamphetamine. *Addiction.* 2009; 104:1085–1099. [PubMed: 19426289]
- Cuevas J, Rodriguez A, Behensky A, Katnik C. Afobazole modulates microglial function via activation of both sigma-1 and sigma-2 receptors. *J Pharmacol Exp Ther.* 2011; 339:161–172. [PubMed: 21715561]
- Czeh M, Gressens P, Kaindl AM. The yin and yang of microglia. *Dev Neurosci.* 2011; 33:199–209. [PubMed: 21757877]
- Derocq JM, Bourrie B, Segui M, Le Fur G, Casellas P. In vivo inhibition of endotoxin-induced pro-inflammatory cytokines production by the sigma ligand SR 31747. *J Pharmacol Exp Ther.* 1995; 272:224–230. [PubMed: 7815336]
- Escubedo E, Chipana C, Perez-Sanchez M, Camarasa J, Pubill D. Methyllycaconitine prevents methamphetamine-induced effects in mouse striatum: involvement of $\alpha 7$ nicotinic receptors. *J Pharmacol Exp Ther.* 2005; 315:658–667. [PubMed: 16076935]
- Escubedo E, Guitart L, Sureda FX, Jimenez A, Pubill D, Pallas M, Camins A, Camarasa J. Microgliosis and down-regulation of adenosine transporter induced by methamphetamine in rats. *Brain Res.* 1998; 814:120–126. [PubMed: 9838075]
- Goncalves J, Martins T, Ferreira R, Milhazes N, Borges F, Ribeiro CF, Malva JO, Macedo TR, Silva AP. Methamphetamine-induced early increase of IL-6 and TNF- α mRNA expression in the mouse brain. *Ann N Y Acad Sci.* 2008; 1139:103–111. [PubMed: 18991854]
- Hall AA, Herrera Y, Ajmo CT Jr. Cuevas J, Pennypacker KR. Sigma receptors suppress multiple aspects of microglial activation. *Glia.* 2009; 57:744–754. [PubMed: 19031439]
- Hashimoto K, Tsukada H, Nishiyama S, Fukumoto D, Kakiuchi T, Iyo M. Protective effects of minocycline on the reduction of dopamine transporters in the striatum after administration of methamphetamine: a positron emission tomography study in conscious monkeys. *Biol Psychiatry.* 2007; 61:577–581. [PubMed: 16712806]
- Hayashi T, Su TP. Sigma-1 receptor chaperones at the ER-mitochondrion interface regulate Ca^{2+} signaling and cell survival. *Cell.* 2007; 131:596–610. [PubMed: 17981125]
- Hebert MA, O'Callaghan JP. Protein phosphorylation cascades associated with methamphetamine-induced glial activation. *Ann N Y Acad Sci.* 2000; 914:238–262. [PubMed: 11085325]
- Kaushal N, Elliott M, Robson MJ, Iyer AK, Rojanasakul Y, Coop A, Matsumoto RR. AC927, a sigma receptor ligand, blocks methamphetamine-induced release of dopamine and generation of reactive oxygen species in NG108-15 cells. *Mol Pharmacol.* 2012a; 81:299–308. [PubMed: 22101517]
- Kaushal, N.; McCurdy, CR.; Matsumoto, RR. 2011 Meeting Planner. Society for Neuroscience; Washington, D.C: 2011a. SN79 attenuates the neurotoxic effect of methamphetamine: in vivo and in vitro studies. 367.03
- Kaushal N, Robson MJ, Vinnakota H, Narayanan S, Avery BA, McCurdy CR, Matsumoto RR. Synthesis and pharmacological evaluation of 6-acetyl-3-(4-(4-(4-fluorophenyl)piperazin-1-yl)butyl)benzo[d]oxazol-2(3H)-one (SN79), a cocaine antagonist, in rodents. *AAPS J.* 2011b; 13:336–346. [PubMed: 21494909]
- Kaushal N, Seminerio MJ, Robson MJ, McCurdy CR, Matsumoto RR. Pharmacological evaluation of SN79, a sigma (σ) receptor ligand, against methamphetamine-induced neurotoxicity in vivo. *Eur Neuropsychopharmacol.* 2012b Epub ahead of print.
- Kaushal N, Seminerio MJ, Shaikh J, Medina MA, Mesangeau C, Wilson LL, McCurdy CR, Matsumoto RR. CM156, a high affinity sigma ligand, attenuates the stimulant and neurotoxic

effects of methamphetamine in mice. *Neuropharmacology*. 2011c; 61:992–1000. [PubMed: 21762711]

Kelly KA, Miller DB, Bowyer JF, O'Callaghan JP. Chronic exposure to corticosterone enhances the neuroinflammatory and neurotoxic responses to methamphetamine. *J Neurochem*. 2012; 122:995–1009. [PubMed: 22776046]

Kita T, Shimada K, Mastunari Y, Wagner GC, Kubo K, Nakashima T. Methamphetamine-induced striatal dopamine neurotoxicity and cyclooxygenase-2 protein expression in BALB/c mice. *Neuropharmacology*. 2000; 39:399–406. [PubMed: 10698006]

Komohara Y, Horlad H, Ohnishi K, Ohta K, Makino K, Hondo H, Yamanaka R, Kajiwarra K, Saito T, Kuratsu J, Takeya M. M2 macrophage/microglial cells induce activation of Stat3 in primary central nervous system lymphoma. *J Clin Exp Hematop*. 2011; 51:93–99. [PubMed: 22104307]

Krasnova IN, Cadet JL. Methamphetamine toxicity and messengers of death. *Brain Res Rev*. 2009; 60:379–407. [PubMed: 19328213]

Kuhn DM, Francescutti-Verbeem DM, Thomas DM. Dopamine quinones activate microglia and induce a neurotoxic gene expression profile: relationship to methamphetamine-induced nerve ending damage. *Ann N Y Acad Sci*. 2006; 1074:31–41. [PubMed: 17105901]

Ladenheim B, Krasnova IN, Deng X, Oyler JM, Poletini A, Moran TH, Huestis MA, Cadet JL. Methamphetamine-induced neurotoxicity is attenuated in transgenic mice with a null mutation for interleukin-6. *Mol Pharmacol*. 2000; 58:1247–1256. [PubMed: 11093760]

LaVoie MJ, Card JP, Hastings TG. Microglial activation precedes dopamine terminal pathology in methamphetamine-induced neurotoxicity. *Exp Neurol*. 2004; 187:47–57. [PubMed: 15081587]

Li B, Mahmood A, Lu D, Wu H, Xiong Y, Qu C, Chopp M. Simvastatin attenuates microglial cells and astrocyte activation and decreases interleukin-1 β level after traumatic brain injury. *Neurosurgery*. 2009; 65:179–185. discussion 185–176. [PubMed: 19574840]

Matsumoto RR, Shaikh J, Wilson LL, Vedam S, Coop A. Attenuation of methamphetamine-induced effects through the antagonism of sigma (σ) receptors: Evidence from in vivo and in vitro studies. *Eur Neuropsychopharmacol*. 2008; 18:871–881. [PubMed: 18755577]

Mizoguchi H, Takuma K, Fukakusa A, Ito Y, Nakatani A, Ibi D, Kim HC, Yamada K. Improvement by minocycline of methamphetamine-induced impairment of recognition memory in mice. *Psychopharmacology (Berl)*. 2008; 196:233–241. [PubMed: 17909751]

Mori T, Hayashi T, Su TP. Compromising sigma-1 receptors at the endoplasmic reticulum render cytotoxicity to physiologically relevant concentrations of dopamine in a nuclear factor-kappaB/Bcl-2-dependent mechanism: potential relevance to Parkinson's disease. *J Pharmacol Exp Ther*. 2012; 341:663–671. [PubMed: 22399814]

Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. *Nat Rev Immunol*. 2008; 8:958–969. [PubMed: 19029990]

Nagai A, Mishima S, Ishida Y, Ishikura H, Harada T, Kobayashi S, Kim SU. Immortalized human microglial cell line: phenotypic expression. *J Neurosci Res*. 2005; 81:342–348. [PubMed: 15957187]

Nguyen EC, McCracken KA, Liu Y, Pouw B, Matsumoto RR. Involvement of sigma (σ) receptors in the acute actions of methamphetamine: receptor binding and behavioral studies. *Neuropharmacology*. 2005; 49:638–645. [PubMed: 15939443]

Niino D, Komohara Y, Murayama T, Aoki R, Kimura Y, Hashikawa K, Kiyasu J, Takeuchi M, Suefuji N, Sugita Y, Takeya M, Ohshima K. Ratio of M2 macrophage expression is closely associated with poor prognosis for angioimmunoblastic T-cell lymphoma (AITL). *Pathol Int*. 2010; 60:278–283. [PubMed: 20403029]

O'Callaghan JP, Sriram K, Miller DB. Defining “neuroinflammation”. *Ann N Y Acad Sci*. 2008; 1139:318–330. [PubMed: 18991877]

Perry VH, Nicoll JA, Holmes C. Microglia in neurodegenerative disease. *Nat Rev Neurol*. 2010; 6:193–201. [PubMed: 20234358]

Robson MJ, Noorbakhsh B, Seminerio MJ, Matsumoto RR. Sigma-1 receptors: potential targets for the treatment of substance abuse. *Curr Pharm Des*. 2012; 18:902–919. [PubMed: 22288407]

Rodvelt KR, Miller DK. Could sigma receptor ligands be a treatment for methamphetamine addiction? *Curr Drug Abuse Rev*. 2010; 3:156–162. [PubMed: 21054260]

- Romanelli F, Smith KM. Clinical effects and management of methamphetamine abuse. *Pharmacotherapy*. 2006; 26:1148–1156. [PubMed: 16863490]
- Ruscher K, Inacio AR, Valind K, Rowshan Ravan A, Kuric E, Wieloch T. Effects of the sigma-1 receptor agonist 1-(3,4-dimethoxyphenethyl)-4-(3-phenylpropyl)-piperazine dihydro-chloride on inflammation after stroke. *PLoS One*. 2012; 7:e45118. [PubMed: 23028794]
- Saijo K, Glass CK. Microglial cell origin and phenotypes in health and disease. *Nat Rev Immunol*. 2011; 11:775–787. [PubMed: 22025055]
- Sekine Y, Ouchi Y, Sugihara G, Takei N, Yoshikawa E, Nakamura K, Iwata Y, Tsuchiya KJ, Suda S, Suzuki K, Kawai M, Takebayashi K, Yamamoto S, Matsuzaki H, Ueki T, Mori N, Gold MS, Cadet JL. Methamphetamine causes microglial activation in the brains of human abusers. *J Neurosci*. 2008; 28:5756–5761. [PubMed: 18509037]
- Seminario MJ, Hansen R, Kaushal N, Zhang HT, McCurdy CR, Matsumoto RR. The evaluation of AZ66, an optimized sigma receptor antagonist, against methamphetamine-induced dopaminergic neurotoxicity and memory impairment in mice. *Int J Neuropsychopharmacol*. 2012:1–12.
- Seminario MJ, Kaushal N, Shaikh J, Huber JD, Coop A, Matsumoto RR. Sigma (σ) receptor ligand, AC927 (N-phenethylpiperidine oxalate), attenuates methamphetamine-induced hyperthermia and serotonin damage in mice. *Pharmacol Biochem Behav*. 2011; 98:12–20. [PubMed: 21130800]
- Smith JA, Das A, Ray SK, Banik NL. Role of pro-inflammatory cytokines released from microglia in neurodegenerative diseases. *Brain Res Bull*. 2012; 87:10–20. [PubMed: 22024597]
- Smith KJ, Butler TR, Prendergast MA. Inhibition of sigma-1 receptor reduces N-methyl-D-aspartate induced neuronal injury in methamphetamine-exposed and -naive hippocampi. *Neurosci Lett*. 2010; 481:144–148. [PubMed: 20600592]
- Tanibuchi Y, Shimagami M, Fukami G, Sekine Y, Iyo M, Hashimoto K. A case of methamphetamine use disorder treated with the antibiotic drug minocycline. *Gen Hosp Psychiatry*. 2010; 32:559–e551. [PubMed: 20851278]
- Thomas DM, Dowgiert J, Geddes TJ, Francescutti-Verbeem D, Liu X, Kuhn DM. Microglial activation is a pharmacologically specific marker for the neurotoxic amphetamines. *Neurosci Lett*. 2004a; 367:349–354. [PubMed: 15337264]
- Thomas DM, Kuhn DM. Attenuated microglial activation mediates tolerance to the neurotoxic effects of methamphetamine. *J Neurochem*. 2005; 92:790–797. [PubMed: 15686480]
- Thomas DM, Walker PD, Benjamins JA, Geddes TJ, Kuhn DM. Methamphetamine neurotoxicity in dopamine nerve endings of the striatum is associated with microglial activation. *J Pharmacol Exp Ther*. 2004b; 311:1–7. [PubMed: 15163680]
- Volkow ND, Chang L, Wang GJ, Fowler JS, Franceschi D, Sedler M, Gatley SJ, Miller E, Hitzemann R, Ding YS, Logan J. Loss of dopamine transporters in methamphetamine abusers recovers with protracted abstinence. *J Neurosci*. 2001a; 21:9414–9418. [PubMed: 11717374]
- Volkow ND, Chang L, Wang GJ, Fowler JS, Leonido-Yee M, Franceschi D, Sedler MJ, Gatley SJ, Hitzemann R, Ding YS, Logan J, Wong C, Miller EN. Association of dopamine transporter reduction with psychomotor impairment in methamphetamine abusers. *Am J Psychiatry*. 2001b; 158:377–382. [PubMed: 11229977]
- Wang GJ, Smith L, Volkow ND, Telang F, Logan J, Tomasi D, Wong CT, Hoffman W, Jayne M, Alia-Klein N, Thanos P, Fowler JS. Decreased dopamine activity predicts relapse in methamphetamine abusers. *Mol Psychiatry*. 2012; 17:918–925. [PubMed: 21747399]
- Yao H, Yang Y, Kim KJ, Bethel-Brown C, Gong N, Funa K, Gendelman HE, Su TP, Wang JQ, Buch S. Molecular mechanisms involving sigma receptor-mediated induction of MCP-1: implication for increased monocyte transmigration. *Blood*. 2010; 115:4951–4962. [PubMed: 20354174]
- Yue X, Qiao D, Wang A, Tan X, Li Y, Liu C, Wang H. CD200 attenuates methamphetamine-induced microglial activation and dopamine depletion. *J Huazhong Univ Sci Technolog Med Sci*. 2012; 32:415–421. [PubMed: 22684568]
- Zhang L, Kitaichi K, Fujimoto Y, Nakayama H, Shimizu E, Iyo M, Hashimoto K. Protective effects of minocycline on behavioral changes and neurotoxicity in mice after administration of methamphetamine. *Prog Neuropsychopharmacol Biol Psychiatry*. 2006; 30:1381–1393. [PubMed: 16839653]

Zhang Z, Zhang ZY, Wu Y, Schluesener HJ. Lesional accumulation of CD163(+) macrophages/microglia in rat traumatic brain injury. *Brain Res.* 2012; 1461:102–110. [PubMed: 22583855]

Highlights

- Methamphetamine (METH) can cause hyperthermia and neurotoxicity
- METH results in microglial activation and cytokine upregulation in the CNS
- SN79, a σ receptor ligand, mitigates METH hyperthermia and neurotoxicity in mice
- SN79 also blocks METH-induced microglial activation and cytokine upregulation
- σ receptors are potential drug development targets to mitigate METH-induced effects

Fig. 1.

Repeated methamphetamine (METH, 5 mg/kg $\times$ 4) treatment increased core body temperature as compared to saline (Sal), an effect mitigated by SN79 (3 mg/kg $\times$ 4) pretreatment. SN79 treatment also displayed no effects on basal body temperature alone. Two-way repeated measures ANOVA, followed by post hoc Bonferroni's multiple comparison tests; *** $p < 0.001$, Sal/Sal vs. Sal/METH; ### $p < 0.001$, Sal/METH vs. SN79/METH. BT=body temperature.

Fig. 2. Methamphetamine treatment (METH, 5 mg/kg $\times$ 4) was found to time-dependently increase *cd68* mRNA expression in the striatum, indicative of microglial cell activation. Two-way ANOVA, followed by Bonferroni's post hoc analysis; * $p < 0.05$, *** $p < 0.001$, Saline vs. METH.

Fig. 3. SN79 (3 mg/kg $\times$ 4) pretreatment attenuated methamphetamine (METH, 5 mg/kg $\times$ 4)-induced increases in striatal CD68 mRNA and protein expression, indicative of a reduction in microglial activation. (A) SN79 treatment resulted in the blockade of METH-induced *cd68* expression in the striatum 24 h post treatment. (B) SN79 pretreatment also blocked protein level increases in CD68 elicited by METH treatment at 72 h. (C) Representative images of CD68 expression for each respective group. Black arrows depict microglia expressing high levels of CD68. 20 $\times$ magnification. One-way ANOVA, followed by post hoc Tukey's multiple comparison tests; * $p < 0.05$, *** $p < 0.001$, Sal/Sal vs. respective group; ### $p < 0.001$, Sal/METH vs. SN79/METH. Sal = saline.

Fig. 4.

SN79 (3 mg/kg $\times$ 4) blocked methamphetamine (METH, 5 mg/kg $\times$ 4)-induced increases in the microglial marker IBA-1 72 h post treatment, confirming an attenuation of METH-induced microglial activation. (A) SN79 treatment blocked METH-induced increases in striatal IBA-1 expression at 72 h. (B) Representative images of striatal IBA-1 expression for each respective group. Black arrows represent microglia with high levels of IBA-1 expression. 20 $\times$ magnification. One-way ANOVA, followed by post hoc Tukey's analysis; * $p < 0.05$, *** $p < 0.001$, Sal/Sal vs. respective group; ### $p < 0.001$, Sal/METH vs. SN79/METH. Sal = saline.

Fig. 5.

None of the drug treatments had a significant effect on levels of M2-type microglia present in the striatum 72 h post treatment (n.s.). Sal = saline, METH = methamphetamine (5 mg/kg $\times$ 4), SN79 = SN79 (3 mg/kg $\times$ 4).

Fig. 6.

Methamphetamine treatment (METH, 5 mg/kg $\times$ 4) increased mRNA expression of interleukin-6 (IL-6) family cytokines in the striatum. (A) METH treatment resulted in significant increases in *il-6* expression at all time points tested. (B) METH also increased striatal *osm* expression at 1.5, 3, 6 and 12 h post treatment. (C) METH further increased *Ilf* expression in the striatum at 1.5, 3, 6 and 12 h post treatment. Two-way ANOVA, followed by post hoc Bonferroni's analysis; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, Saline vs. METH.

Fig. 7.

SN79 pretreatment (3 mg/kg $\times$ 4) blocked methamphetamine (METH, 5 mg/kg $\times$ 4)-induced increases in mRNA expression of IL-6 family cytokines in the striatum 6 h post treatment.

(A) SN79 attenuated METH-induced increases in *il-6* expression. (B) METH treatment significantly increased striatal *osm* expression, an effect mitigated by SN79 treatment. (C) SN79 attenuated METH-induced increases in *lif* expression. One-way ANOVA, followed by post hoc Tukey's multiple comparison tests; *** $p < 0.001$, Sal/Sal vs. Sal/METH; ## $p < 0.01$, ### $p < 0.001$, Sal/METH vs. SN79/METH. Sal = saline.