Published in final edited form as:

Eur J Pharmacol. 2012 September 15; 691(0): 103–109. doi:10.1016/j.ejphar.2012.07.029.

Sigma receptor antagonists attenuate acute methamphetamine-induced hyperthermia by a mechanism independent of IL-1 β mRNA expression in the hypothalamus

Michael J. Seminerio^a, Matthew J. Robson^a, Christopher R. McCurdy^b, and Rae R. Matsumoto^{a,*}

^aDepartment of Basic Pharmaceutical Sciences, School of Pharmacy, West Virginia University, Morgantown, WV 26506, USA

^bDepartment of Medicinal Chemistry and Department of Pharmacology, School of Pharmacy, University of Mississippi, University, MS 38677, USA

Abstract

Methamphetamine is currently one of the most widely abused drugs worldwide, with hyperthermia being a leading cause of death in methamphetamine overdose situations. Methamphetamine-induced hyperthermia involves a variety of cellular mechanisms, including increases in hypothalamic interleukin-1 beta (IL-1 β) expression. Methamphetamine also interacts with sigma receptors and previous studies have shown that sigma receptor antagonists mitigate many of the behavioral and physiological effects of methamphetamine, including hyperthermia. The purpose of the current study was to determine if the attenuation of methamphetamine-induced hyperthermia by the sigma receptor antagonists, AZ66 and SN79, is associated with a concomitant attenuation of IL-1 β mRNA expression, particularly in the hypothalamus. Methamphetamine produced dose and time-dependent increases in core body temperature and IL-1 β mRNA expression in the hypothalamus, striatum, and cortex in male, Swiss Webster mice. Pretreatment with the sigma receptor antagonists, AZ66 and SN79, significantly attenuated methamphetamine-induced hyperthermia, but further potentiated IL-1 β mRNA in the mouse hypothalamus when compared to animals treated with methamphetamine alone. These findings suggest sigma receptor antagonists attenuate methamphetamine-induced hyperthermia through a different mechanism from that involved in the modulation of hypothalamic IL-1 β mRNA expression.

Keywords

Hyperthermia; Hypothalamus; Interleukin-1 β ; Methamphetamine; Sigma Receptor

1. Introduction

Methamphetamine has long been a drug used for recreational purposes with an estimated 16 million users worldwide (United Nations, 2007). Recent reports indicate methamphetamine

© 2012 Elsevier B.V. All rights reserved.

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

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

abuse has eclipsed that of cocaine and heroin on a global scale (United Nations, 2007). Following toxic doses of methamphetamine, life-threatening increases in body temperature occur, and both clinical reports and animal studies suggest methamphetamine-induced lethality is closely related to hyperthermia, and may be a primary cause of death (Bowyer et al., 1994; Davidson et al., 2001). However, the mechanisms by which methamphetamine produces its effects, particularly temperature deregulation, remain poorly understood.

Earlier studies exploring the mechanisms of methamphetamine-induced hyperthermia have reported that following administration of methamphetamine, the proinflammatory cytokine interleukin-1 beta (IL-1 β) increases in the thermoregulatory region of the brain, the hypothalamus (Bandtlow et al., 1990; Bowyer et al., 1994; Yamaguchi et al., 1991). IL-1 is an endogenous pyrogen (Kluger, 1991; Leon, 2002) that is released from activated microglial cells (Wang et al., 2008b). Methamphetamine has been shown to activate microglial cells in vivo, at doses that result in hyperthermia (Kuhn et al., 2006; Sekine et al., 2008), suggesting that a release of IL-1 may be responsible for changes in core body temperature produced by methamphetamine.

Methamphetamine also interacts with sigma receptors at physiologically relevant concentrations, and selective sigma receptor antagonists can attenuate methamphetamine-induced hyperthermia in experimental animals (Matsumoto et al., 2008; Nguyen et al., 2005; Rodvelt and Miller, 2010; Seminerio et al., 2011). Interestingly, sigma receptors are found on microglial cells (Gekker et al., 2006), and sigma receptor antagonists have been shown to attenuate microglial activation, by inhibiting both membrane ruffling and migration (Cuevas et al., 2011; Hall et al., 2009). The ability of sigma receptor antagonists to mitigate methamphetamine-induced hyperthermia and modulate microglial activation led to the hypothesis that the ability of these ligands to attenuate hyperthermic responses to methamphetamine may stem from the modulation of IL-1 β in the hypothalamus.

The purpose of the current study was to determine if sigma receptor antagonists can attenuate acute increases in body temperature following a bolus dose of methamphetamine and whether these protective effects occur through modulation of IL-1 β mRNA expression in the hypothalamus. IL-1 β mRNA expression was measured in the current study, instead of actual cytokine levels, to ensure that increases detected were from the brain region of interest and not the systemic circulation. This was important because methamphetamine has been shown to increase the release of proinflammatory cytokines such as IL-1 β in the periphery (Buchanan et al., 2010).

In addition to determining the effects of methamphetamine on IL-1 β mRNA expression in the brain, two sigma receptor antagonists, AZ66 (3-(4-(4-cyclohexylpiperazin-1-yl)pentyl)-6-fluorobenzo[d]thiazol-2(3H)-one) and SN79 (6-acetyl-3-(4-(4-(4-fluorophenyl)piperazin-1-yl)butyl)benzo[d]oxazol-2(3H)-one), were evaluated to determine if their ability to attenuate methamphetamine-induced hyperthermia arises from an ability to attenuate methamphetamine-induced increases in hypothalamic IL-1 β mRNA levels. These two sigma receptor ligands were selected because both have been previously shown to exhibit profiles consistent with antagonist actions, such as the ability to mitigate methamphetamine-induced hyperthermia and neurotoxicity in a different experimental paradigm, and to also have favorable pharmacokinetic profiles amenable for further drug development (Kaushal et al., 2011a; Kaushal et al., 2011b; Seminerio et al., 2012).

2. Materials and methods

2.1. Drugs and reagents

1 (+)-Methamphetamine hydrochloride was purchased from Sigma-Aldrich (St. Louis, MO) and sterile saline solution was purchased from Teknova (Hollister, CA). The sigma receptor ligands, AZ66 and SN79, were synthesized as previously described (Kaushal et al., 2011b; Seminario et al., 2012). All drug solutions were made with saline, and the solution volumes were administered relative to body weight (0.1 ml/10 g).

2.1. Animals

Male, Swiss Webster mice (24–28 g, Harlan, Indianapolis, IN and Frederick, MD) were used for all experiments. Mice were housed five per cage on a 12:12 hr light dark cycle with both food and water *ad libitum*. Mice were allowed to acclimate to the animal housing facility for at least one week prior to being randomized for use in experiments. They were then allowed to habituate for at least one hour to the testing facility immediately prior to the initiation of all experiments. All procedures were performed as approved by the Institutional Animal Care and Use Committee at the West Virginia University Health Sciences Center.

2.2. In vivo experiments

Validation experiments were conducted on naïve animals to confirm the hypothalamus was properly dissected with minimal contamination from the pituitary gland. Male, Swiss Webster mice were sacrificed by decapitation and the hypothalamus, pituitary gland and prefrontal cortex were removed and immediately frozen in liquid nitrogen for later analysis. The mRNA for thyroid releasing hormone (TRH), a hormone specifically expressed in the hypothalamus, was measured via quantitative real-time polymerase chain reaction (PCR) in both the hypothalamus and pituitary gland tissues. In addition, the mRNA for thyroid stimulating hormone (TSH), a hormone specifically expressed in the pituitary gland, was measured in both tissues. The pfrontal cortex was utilized for normalization and negative control purposes.

To determine the effects of acute methamphetamine treatment on body temperature and hypothalamic mRNA IL-1 β expression, dose and time response studies were conducted. Baseline, core body temperature measurements were determined using a rectal probe (Physitemp, Clifton, NJ) as previously described (Seminario et al., 2011a). Animals were then pretreated with saline (0.1 ml/10 g, i.p.) followed 15 min later with either saline (0.1 ml/10 g, i.p.) or methamphetamine (25, 35, 45 mg/kg, i.p.). Core body temperature measurements were then recorded every 15 min. Animals were sacrificed 15, 30, or 45 min after the treatments, and brain samples were collected from each animal. Three different brain regions were evaluated for IL-1 β mRNA expression: hypothalamus, striatum, and cortex. The hypothalamus was selected due to its role in temperature regulation and previous studies showing methamphetamine increases mRNA IL-1 β expression in this brain region (Bandtlow et al., 1990; Bowyer et al., 1994; Yamaguchi et al., 1991). The other two brain regions were chosen for comparison because they do not have important roles in thermoregulation, but are key regions of the brain in which methamphetamine produces neurotoxicity (Cadet et al., 2007). Samples were flash frozen using liquid nitrogen and subsequently stored at –80 °C for later analysis.

Experiments utilizing sigma receptor antagonists were conducted in a similar fashion to those described above. A sigma receptor antagonist (AZ66 or SN79) was used as a pretreatment (10 or 3 mg/kg, i.p., respectively) 15 min prior to administration of either saline (0.1 ml/10 g, i.p.) or methamphetamine (35 mg/kg, i.p.). Methamphetamine (35 mg/kg, i.p.) was used because in preliminary studies, it increased hypothalamic IL-1 β mRNA to

similar levels as higher doses of methamphetamine, but with a lower incidence of lethality. AZ66 (10 mg/kg) and SN79 (3 mg/kg) were used because previous studies have shown at these doses, there is no significant effect in the absence of methamphetamine; however, they exhibit neuroprotective effects when administered prior to methamphetamine (Seminario et al., 2012; Kaushal et al., 2011a). Core body temperatures were recorded immediately prior to the initiation of the experiments (baseline readings) and every 15 min following the drug treatments. Animals pretreated with sigma receptor antagonists were sacrificed 45 min after receiving their respective treatments with either saline or methamphetamine. The 45 min time point ensured significant increases in IL-1 β mRNA expression in the presence of methamphetamine alone. Tissue samples were collected as described above and stored for later analysis.

2.3. Quantitative real-time PCR

Quantitative real-time PCR was utilized to determine IL-1 β mRNA expression in the hypothalamus, striatum, and cortex. Total RNA was extracted from collected samples using Trizol (Invitrogen, Carlsbad, CA) as per manufacturer's instructions. Reverse transcription and quantitative real-time PCR were conducted as previously described (Xu et al., 2012). TaqMan primers (Applied Biosystems, Foster City, CA) utilized for quantitative real-time PCR included: IL-1 β (Mm00434228_m1), TSHb (Mm01308709_g1) and TRH (Mm01182425_g1). 18s (Hs99999901_s1; Applied Biosystems, Foster City, CA) was used as the endogenous control. Data was analyzed using the $\Delta\Delta C_t$ method. The threshold value was set to 0.2 for all experiments and the threshold cycle (C_t) of each gene was normalized to the 18s endogenous control.

2.4. Statistical analysis

Data was analyzed using GraphPad Prism 4.0 (San Diego, CA). The hypothalamus validation quantitative real-time PCR data was analyzed using unpaired t-tests. Core body temperature recordings and quantitative real-time PCR data for the remaining experiments were analyzed using one-way ANOVA, followed where applicable by either post-hoc Dunnett's tests (for comparisons to control) or Tukey's multiple comparison tests (for pairwise comparisons). Pearson's analysis was used for correlations. $P < 0.05$ was considered significant for all statistical analyses.

3. Results

3.1. Hypothalamus validation

An unpaired t-test confirmed a significant difference in TRH mRNA expression in the hypothalamus (mean fold change in TRH mRNA = 670, normalized to prefrontal cortex) as compared to the pituitary gland (mean fold change in TRH mRNA = 2.03, normalized to prefrontal cortex) ($t = 2.62$, $P < 0.0001$). An unpaired t-test also confirmed a significant difference in TSH mRNA expression in the pituitary gland (mean fold change in TSH mRNA = 2968, normalized to prefrontal cortex) as compared to the hypothalamus (mean fold change in TSH mRNA = 2.16, normalized to prefrontal cortex) ($t = 6.11$, $P < 0.0001$).

3.2. Methamphetamine time course and dose response on body temperature

One way ANOVA showed methamphetamine produced a significant increase in body temperature at each of the time points evaluated (15 min: $F(3,29) = 7.91$, $P < 0.05$; 30 min: $F(3,29) = 30.76$, $P < 0.001$; 45 min: $F(3,29) = 52.19$, $P < 0.001$) (Fig. 1). Post-hoc Dunnett's tests confirmed that methamphetamine produced significant increases in body temperature as compared to saline controls at the 15 min time point (45 mg/kg: $q = 9.16$, $P < 0.01$), 30 min time point (25 mg/kg: $q = 6.35$, $P < 0.01$; 35 mg/kg: $q = 6.63$, $P < 0.01$; 45 mg/kg: $q = 9.27$,

$P<0.01$), and 45 min time point (25 mg/kg: $q=7.78$, $P<0.01$; 35 mg/kg: $q=8.62$, $P<0.01$; 45 mg/kg: $q=12.17$, $P<0.001$). Since methamphetamine produced the most significant changes at the 45 min time point, it was used in all subsequent studies.

3.3. Methamphetamine dose response on IL-1 β mRNA expression

One way ANOVA showed a significant increase in IL-1 β mRNA expression following methamphetamine administration in the mouse hypothalamus ($F(3,39)=5.86$, $P<0.01$) (Fig. 2a), striatum ($F(3,23)=4.72$, $P<0.05$) (Fig. 2b), and cortex ($F(3,23)=3.41$, $P<0.05$) (Fig. 2c). Post-hoc Dunnett's tests confirmed that both 35 mg/kg and 45 mg/kg of methamphetamine caused a significant increase in IL-1 β mRNA in the mouse hypothalamus compared to saline controls ($q=3.13$, $P<0.01$; $q=3.41$, $P<0.01$, respectively). Furthermore, post-hoc Dunnett's tests revealed significant differences compared to saline in the striatum and cortex (striatum: 35 mg/kg, $q=2.92$, $P<0.05$; 45 mg/kg, $q=3.22$, $P<0.05$; cortex: 45 mg/kg, $q=3.04$, $P<0.05$). Lastly, a Pearson's correlation demonstrated no significant relationship between body temperature and IL-1 β mRNA following saline or methamphetamine (25, 35, and 45 mg/kg, i.p.) administration in the hypothalamus ($r=0.042$, $P>0.05$, Fig. 3).

3.4. Effects of sigma receptor antagonists

One-way ANOVA showed significant differences in core body temperature between the groups following pretreatment with AZ66 ($F(3,15)=4.52$, $P<0.05$, Fig. 4a) or SN79 ($F(3,15)=6.70$, $P<0.01$, Fig. 4b). Post-hoc Tukey's tests confirmed methamphetamine produced a significant increase in body temperature at each of the time points (15 min: $q=4.47$, $P<0.05$; 30 min: $q=15.20$, $P<0.001$; 45 min: $q=14.13$, $P<0.001$) and pretreatment with AZ66 attenuated methamphetamine-induced hyperthermia (15 min: $q=5.05$, $P<0.05$; 30 min: $q=8.99$, $P<0.001$; 45 min: $q=8.52$, $P<0.001$, Fig. 4a). Similarly, pretreatment with SN79 significantly attenuated methamphetamine-induced hyperthermia (15 min: $q=14.52$, $P<0.001$; 30 min: $q=15.81$, $P<0.001$; 45 min: $q=8.00$, $P<0.001$, Fig. 4b). Both sigma compounds had no effects on their own on body temperature when compared to saline controls at any of the time points tested (post-hoc Tukey's tests, $P>0.05$).

One-way ANOVA showed significant differences in hypothalamic IL-1 β mRNA expression (45 min after 35 mg/kg methamphetamine) between the groups in the presence and absence of AZ66 ($F(3,16)=22.47$, $P<0.001$, Fig. 5a) or SN79 ($F(3,16)=146.80$, $P<0.001$, Fig. 5b). Post-hoc Tukey's tests confirmed that pretreatment with AZ66 or SN79 significantly potentiated methamphetamine-induced increases in hypothalamic IL-1 β mRNA expression ($q=4.23$, $P<0.05$; $q=13.98$, $P<0.001$, respectively). Neither sigma compound had any effect on hypothalamic IL-1 β expression on their own when compared to saline-treated controls (post-hoc Tukey's tests, $P>0.05$).

4. Discussion

The results of these experiments demonstrate a dose- and time-dependent increase in body temperature following an acute bolus administration of methamphetamine. Pretreatment with AZ66 or SN79 significantly attenuated the increases in body temperature, providing the first report that shows pretreatment with sigma receptor antagonists can attenuate hyperthermia following an acute, large bolus dose of methamphetamine. Consistent with earlier reports showing the ability of sigma receptor antagonists to mitigate methamphetamine-induced hyperthermia in a repeated dosing paradigm (Kaushal et al., 2011c; Seminario et al., 2011), this report further emphasizes a modulatory role of sigma receptors in the hyperthermic effects of methamphetamine. This is an important finding as it pertains to methamphetamine overdose situations which are commonly seen in emergency room scenarios (Albertson et al., 1999; Davidson et al., 2001; Sprague et al., 2004).

Methamphetamine also produces significant increases in IL-1 β mRNA expression in the mouse hypothalamus, a key brain region responsible for temperature regulation, as well as in the striatum and the cortex. Methamphetamine has long been known to cause marked increases of IL-1 β mRNA expression in the hypothalamus (Halladay et al., 2003; Yamaguchi et al., 1991) and it has been hypothesized that this increase may be one mechanism by which methamphetamine elicits its temperature deregulation (Bowyer et al., 1994; Yamaguchi et al., 1991). Data presented within this report, however, appear to contradict this hypothesis, as pretreatment with both AZ66 and SN79 fully attenuated methamphetamine-induced increases in core body temperature, yet significantly potentiated methamphetamine-induced expression of hypothalamic IL-1 β mRNA. It is conceivable that using other doses and time points may produce different results from what we observed herein; however, further supporting our conclusions is the lack of correlation between core body temperature and IL-1 β mRNA levels in the hypothalamus in methamphetamine-treated animals. Moreover, previous studies demonstrated that pretreatment with IL-1 receptor antagonists were unable to fully attenuate methamphetamine-induced or 3,4-methylenedioxymethamphetamine (MDMA)-induced hyperthermia (Bowyer et al., 1994; Orio et al., 2004). Together, the body of data suggests a minimal role for IL-1 β in the ability of methamphetamine to cause significant hyperthermia or the ability of sigma receptor antagonists to mitigate methamphetamine-induced hyperthermia.

Two implications of this are: 1) other mechanisms besides IL-1 likely mediate the ability of sigma receptor antagonists to attenuate methamphetamine-induced hyperthermia, and 2) IL-1 increases are important for effects of methamphetamine other than hyperthermia. Both of these issues are addressed in the paragraphs that follow.

A well characterized source for IL-1 β release is activated microglia cells (Bandtlow et al., 1990; Wang et al., 2008a). Previous studies have shown that suppression of activated microglia-induced release of interleukin-1 in addition to interleukin-6 and chemokines may, in part, contribute to the neuroprotective effects of hypothermia (Aibiki et al., 1999; Marion et al., 1997; Matsui et al., 2011). Sigma receptors are expressed on microglial cells (Gekker et al., 2006) and sigma receptor antagonists have been shown to inhibit microglial activation thereby modulating the release of cytokines (Hall et al., 2009). In separate studies, sigma receptor ligands have been shown to modulate a variety of chemokines and cytokines that affect inflammation (Kvaratskhelia et al., 2009; Yao et al., 2010), suggesting a potential mechanism through which sigma ligands may mitigate inflammatory processes that are activated by methamphetamine.

In addition to the changes observed in the hypothalamus, the experiments in this study also showed an increase in IL-1 β mRNA in the mouse striatum and cortex following acute, bolus administration of methamphetamine. Methamphetamine-induced neurotoxicity has been reported in both of these brain regions (Gross et al., 2011; Li et al., 2008; Matsumoto et al., 2008). Moreover, previously studied sigma receptor antagonists have been shown to attenuate the neurotoxicity of methamphetamine in vivo, as measured by alterations in dopamine and dopamine transporter levels in the striatum (Kaushal et al., 2011c; Matsumoto et al., 2008; Seminario et al., 2011). Together, the studies suggest that the changes in IL-1 β mRNA expression in the brain may have greater physiological relevance for methamphetamine-induced neurotoxicity, and protection by sigma receptor antagonists.

IL-1 β and its proinflammatory properties have classically been thought to be detrimental in the effects of several neurotoxins including amphetamine derivatives (Goncalves et al., 2008). However, recent reports have shown that initiation of inflammation can provide beneficial effects against amphetamine neurotoxicity. Systemic lipopolysaccharide (LPS) administration, which results in striatal increases in IL-1 β , either 3 days before or 2 hrs after

a neurotoxic administration of methamphetamine, has been shown to mitigate striatal dopamine reductions elicited by methamphetamine treatment (Lai et al., 2009). Additionally, administering low doses of amphetamines (methamphetamine and MDMA) prior to a neurotoxic regimen of each respective drug can result in protection from the neurotoxic consequences (Bhide et al., 2009; Cadet et al., 2009; Hodges et al., 2011). Administration of a low dose of MDMA in rodents prior to a neurotoxic dose of MDMA has also been shown to produce increases in IL-1 β levels, which can protect against the serotonergic neurotoxicity associated with MDMA (Mayado et al., 2011). Although these results provide evidence that IL-1 β may provide protection against the neurotoxic actions of MDMA, whether IL-1 β confers similar protective properties in our experiments utilizing methamphetamine has yet to be determined.

The dopaminergic and serotonergic systems also appear to have an important role in thermoregulation. Methamphetamine produces an increase in the release of dopamine and serotonin (Phelps et al., 2010). Both of these monoamines are involved in temperature regulation in the CNS (Cannon and Nedergaard, 2004; Salmi and Ahlenius, 1998; Salmi et al., 1993) and may have roles in methamphetamine-induced hyperthermia (Bowyer et al., 1994; Rusyniak et al., 2008). Earlier studies have shown that sigma receptor ligands modulate the release of both dopamine and serotonin under a variety of experimental conditions (Gonzalez and Werling, 1997; Liu et al., 2001; Nuwayhid and Werling, 2003; Takahashi et al., 2001). In addition, the selective sigma receptor putative antagonist, AC927 (N-phenethylpiperidine oxalate), reduces methamphetamine-induced dopamine release and hyperthermia (Kaushal et al., 2012; Seminerio et al., 2011).

Similar to dopamine, serotonin has a distinctive role in thermoregulation. Previous data have shown that changes in neuronal serotonin levels correlate with changes in brain and core body temperatures (Salmi and Ahlenius, 1998; Schwartz et al., 1995). Methamphetamine facilitates serotonin release in the nerve terminal (Ago et al., 2006; Kuczenski et al., 1995) which can lead to increases in body temperature (Fukumura et al., 1998; Numachi et al., 2007). Since sigma receptors are found in the hypothalamus (Bouchard and Quirion, 1997), it is conceivable that sigma receptor-induced modulation of monoamine release in the hypothalamus may be contributing to methamphetamine-induced temperature deregulation.

Overall, our results indicate that sigma receptor antagonists mitigate many effects of methamphetamine, including temperature deregulation. Additional studies will need to be undertaken which target entities other than IL-1 to delineate the intervening cellular mechanism behind the alterations in body temperature. Moreover, studies to determine the physiological relevance of increases in IL-1 mRNA expression following methamphetamine in regions of the brain such as the striatum and cortex are needed. These studies may provide valuable insight into the precise mechanisms by which methamphetamine elicits hyperthermia, as well as neurotoxic responses.

Acknowledgments

These studies were supported by grants from the National Institute on Drug Abuse (DA013978, DA023205). Michael Seminerio was supported by an institutional training grant from the National Institutes of General Medical Sciences (T32 GM081741).

References

- Ago Y, Nakamura S, Uda M, Kajii Y, Abe M, Baba A, Matsuda T. Attenuation by the 5-HT1A receptor agonist osetozotan of the behavioral effects of single and repeated methamphetamine in mice. *Neuropharmacology*. 2006; 51:914–922. [PubMed: 16863654]

- Aibiki M, Maekawa S, Nishiyama T, Seki K, Yokono S. Activated cytokine production in patients with accidental hypothermia. *Resuscitation*. 1999; 41:263–268. [PubMed: 10507712]
- Albertson TE, Derlet RW, Van Hoozen BE. Methamphetamine and the expanding complications of amphetamines. *West J Med*. 1999; 170:214–219. [PubMed: 10344175]
- Bandtlow CE, Meyer M, Lindholm D, Spranger M, Heumann R, Thoenen H. Regional and cellular codistribution of interleukin 1 beta and nerve growth factor mRNA in the adult rat brain: possible relationship to the regulation of nerve growth factor synthesis. *J Cell Biol*. 1990; 111:1701–1711. [PubMed: 2211833]
- Bhide NS, Lipton JW, Cunningham JI, Yamamoto BK, Gudelsky GA. Repeated exposure to MDMA provides neuroprotection against subsequent MDMA-induced serotonin depletion in brain. *Brain Res*. 2009; 1286:32–41. [PubMed: 19555677]
- Bouchard P, Quirion R. [³H]1,3-di(2-tolyl)guanidine and [³H](+)-pentazocine binding sites in the rat brain: autoradiographic visualization of the putative sigma₁ and sigma₂ receptor subtypes. *Neuroscience*. 1997; 76:467–477. [PubMed: 9015331]
- 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]
- Buchanan JB, Sparkman NL, Johnson RW. Methamphetamine sensitization attenuates the febrile and neuroinflammatory response to a subsequent peripheral immune stimulus. *Brain Behav Immun*. 2010; 24:502–511. [PubMed: 20035859]
- Cadet JL, Krasnova IN, Jayanthi S, Lyles J. Neurotoxicity of substituted amphetamines: molecular and cellular mechanisms. *Neurotox Res*. 2007; 11:183–202. [PubMed: 17449459]
- Cadet JL, McCoy MT, Cai NS, Krasnova IN, Ladenheim B, Beauvais G, Wilson N, Wood W, Becker KG, Hodges AB. Methamphetamine preconditioning alters midbrain transcriptional responses to methamphetamine-induced injury in the rat striatum. *PLoS One*. 2009; 4:e7812. [PubMed: 19915665]
- Cannon B, Nedergaard J. Brown adipose tissue: function and physiological significance. *Physiol Rev*. 2004; 84:277–359. [PubMed: 14715917]
- 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]
- Davidson C, Gow AJ, Lee TH, Ellinwood EH. Methamphetamine neurotoxicity: necrotic and apoptotic mechanisms and relevance to human abuse and treatment. *Brain Res Brain Res Rev*. 2001; 36:1–22. [PubMed: 11516769]
- Fukumura M, Cappon GD, Pu C, Broening HW, Vorhees CV. A single dose model of methamphetamine-induced neurotoxicity in rats: effects on neostriatal monoamines and glial fibrillary acidic protein. *Brain Res*. 1998; 806:1–7. [PubMed: 9739098]
- Gekker G, Hu S, Sheng WS, Rock RB, Lokensgard JR, Peterson PK. Cocaine- induced HIV-1 expression in microglia involves sigma-1 receptors and transforming growth factor-beta1. *Int Immunopharmacol*. 2006; 6:1029–1033. [PubMed: 16644490]
- 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-alpha mRNA expression in the mouse brain. *Ann N Y Acad Sci*. 2008; 1139:103–111. [PubMed: 18991854]
- Gonzalez GM, Werling LL. Release of [³H]dopamine from guinea pig striatal slices is modulated by sigma₁ receptor agonists. *Naunyn Schmiedebergs Arch Pharmacol*. 1997; 356:455–461. [PubMed: 9349631]
- Gross NB, Duncker PC, Marshall JF. Striatal dopamine D1 and D2 receptors: Widespread influences on methamphetamine-induced dopamine and serotonin neurotoxicity. *Synapse*. 2011; 65:1144–1155. [PubMed: 21584865]
- 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]
- Halladay AK, Kusnecov A, Michna L, Kita T, Hara C, Wagner GC. Relationship between methamphetamine-induced dopamine release, hyperthermia, self-injurious behaviour and long

term dopamine depletion in BALB/c and C57BL/6 mice. *Pharmacol Toxicol.* 2003; 93:33–41. [PubMed: 12828572]

Hodges AB, Ladenheim B, McCoy MT, Beauvais G, Cai N, Krasnova IN, Cadet JL. Long-term protective effects of methamphetamine preconditioning against single-day methamphetamine toxic challenges. *Curr Neuropharmacol.* 2011; 9:35–39. [PubMed: 21886558]

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.* 2012; 81:299–308. [PubMed: 22101517]

Kaushal N, McCurdy CR, Matsumoto RR. SN79 attenuates the neurotoxic effects of methamphetamine: In vivo and in vitro studies. Society for Neuroscience 2011 Abstract no. 2011a; 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, Seminario 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]

Kluger MJ. Fever: role of pyrogens and cryogens. *Physiol Rev.* 1991; 71:93–127. [PubMed: 1986393]

Kuczenski R, Segal DS, Cho AK, Melega W. Hippocampus norepinephrine, caudate dopamine and serotonin, and behavioral responses to the stereoisomers of amphetamine and methamphetamine. *J Neurosci.* 1995; 15:1308–1317. [PubMed: 7869099]

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]

Kvaratskhelia E, Maisuradze E, Dabrundashvili NG, Natsvlishvili N, Zhuravliova E, Mikeladze DG. N-methyl-D-aspartate and sigma-ligands change the production of interleukins 8 and 10 in lymphocytes through modulation of the NMDA glutamate receptor. *Neuroimmunomodulation.* 2009; 16:201–207. [PubMed: 19246943]

Lai YT, Tsai YP, Cherg CG, Ke JJ, Ho MC, Tsai CW, Yu L. Lipopolysaccharide mitigates methamphetamine-induced striatal dopamine depletion via modulating local TNF-alpha and dopamine transporter expression. *J Neural Transm.* 2009; 116:405–415. [PubMed: 19271121]

Leon LR. Invited review: cytokine regulation of fever: studies using gene knockout mice. *J Appl Physiol.* 2002; 92:2648–2655. [PubMed: 12015385]

Li X, Wang H, Qiu P, Luo H. Proteomic profiling of proteins associated with methamphetamine-induced neurotoxicity in different regions of rat brain. *Neurochem Int.* 2008; 52:256–264. [PubMed: 17904249]

Liu X, Nuwayhid S, Christie MJ, Kassiou M, Werling LL. Trishomocubanes: novel sigma-receptor ligands modulate amphetamine-stimulated [³H]dopamine release. *Eur J Pharmacol.* 2001; 422:39–45. [PubMed: 11430911]

Marion DW, Penrod LE, Kelsey SF, Obrist WD, Kochanek PM, Palmer AM, Wisniewski SR, DeKosky ST. Treatment of traumatic brain injury with moderate hypothermia. *N Engl J Med.* 1997; 336:540–546. [PubMed: 9023090]

Matsui T, Motoki Y, Inomoto T, Miura D, Kato Y, Suenaga H, Hino K, Nojima J. Temperature-related effects of adenosine triphosphate-activated microglia on pro-inflammatory factors. *Neurocrit Care.* 2011 Epub ahead of print.

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]

Mayado A, Torres E, Gutierrez-Lopez MD, Colado MI, O'Shea E. Increased interleukin-1beta levels following low dose MDMA induces tolerance against the 5-HT neurotoxicity produced by challenge MDMA. *J Neuroinflammation.* 2011; 8:165. [PubMed: 22114930]

- 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]
- Numachi Y, Ohara A, Yamashita M, Fukushima S, Kobayashi H, Hata H, Watanabe H, Hall FS, Lesch KP, Murphy DL, Uhl GR, Sora I. Methamphetamine-induced hyperthermia and lethal toxicity: role of the dopamine and serotonin transporters. *Eur J Pharmacol*. 2007; 572:120–128. [PubMed: 17673199]
- Nuwayhid SJ, Werling LL. Sigma1 receptor agonist-mediated regulation of N-methyl-D-aspartate-stimulated [3 H]dopamine release is dependent upon protein kinase C. *J Pharmacol Exp Ther*. 2003; 304:364–369. [PubMed: 12490613]
- Orio L, O'Shea E, Sanchez V, Pradillo JM, Escobedo I, Camarero J, Moro MA, Green AR, Colado MI. 3,4-Methylenedioxymethamphetamine increases interleukin-1 levels and activates microglia in rat brain: studies on the relationship with acute hyperthermia and 5-HT depletion. *J Neurochem*. 2004; 89:1445–1453. [PubMed: 15189347]
- Phelps G, Speaker HA, Sabol KE. Relationship between methamphetamine-induced behavioral activation and hyperthermia. *Brain Res*. 2010; 1357:41–52. [PubMed: 20709036]
- Rodvelt KR, Miller DK. Could sigma receptor ligands be a treatment for methamphetamine addiction? *Curr Drug Abuse Rev*. 2010; 3:156–162. [PubMed: 21054260]
- Rusyniak DE, Ootsuka Y, Blessing WW. When administered to rats in a cold environment, 3,4-methylenedioxymethamphetamine reduces brown adipose tissue thermogenesis and increases tail blood flow: effects of pretreatment with 5-HT1A and dopamine D2 antagonists. *Neuroscience*. 2008; 154:1619–1626. [PubMed: 18534763]
- Salmi P, Ahlenius S. Evidence for functional interactions between 5-HT1A and 5-HT2A receptors in rat thermoregulatory mechanisms. *Pharmacol Toxicol*. 1998; 82:122–127. [PubMed: 9553989]
- Salmi P, Jimenez P, Ahlenius S. Evidence for specific involvement of dopamine D1 and D2 receptors in the regulation of body temperature in the rat. *Eur J Pharmacol*. 1993; 236:395–400. [PubMed: 8102971]
- Schwartz PJ, Wehr TA, Rosenthal NE, Bartko JJ, Oren DA, Luetke C, Murphy DL. Serotonin and thermoregulation. Physiologic and pharmacologic aspects of control revealed by intravenous m-CPP in normal human subjects. *Neuropsychopharmacology*. 1995; 13:105–115. [PubMed: 8597522]
- 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, 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]
- Seminario MJ, Robson MJ, Abdelazeem AH, Mesangeau C, Jamalapuram S, Avery BA, McCurdy CR, Matsumoto RR. Synthesis and pharmacological characterization of a novel sigma receptor ligand with improved metabolic stability and antagonistic effects against methamphetamine. *AAPS J*. 2012; 14:43–51. [PubMed: 22183188]
- Sprague JE, Mallett NM, Rusyniak DE, Mills E. UCP3 and thyroid hormone involvement in methamphetamine-induced hyperthermia. *Biochem Pharmacol*. 2004; 68:1339–1343. [PubMed: 15345323]
- Takahashi S, Horikomi K, Kato T. MS-377, a novel selective sigma1 receptor ligand, reverses phencyclidine-induced release of dopamine and serotonin in rat brain. *Eur J Pharmacol*. 2001; 427:211–219. [PubMed: 11567651]
- United Nations. United Nations Office on Drugs and Crime World Drug Report Analysis. Vol. Volume 1. Vienna: 2007.
- Wang G, Zhang J, Li W, Xin G, Su Y, Gao Y, Zhang H, Lin G, Jiao X, Li K. Apoptosis and proinflammatory cytokine responses of primary mouse microglia and astrocytes induced by human H1N1 and avian H5N1 influenza viruses. *Cell Mol Immunol*. 2008a; 5:113–120. [PubMed: 18445341]

- Wang P, Rothwell NJ, Pinteaux E, Brough D. Neuronal injury induces the release of pro-interleukin-1 β from activated microglia in vitro. *Brain Res.* 2008b; 1236:1–7. [PubMed: 18722361]
- Xu YT, Robson MJ, Szeszel-Fedorowicz W, Patel D, Rooney R, McCurdy CR, Matsumoto RR. CM156, a sigma receptor ligand, reverses cocaine-induced place conditioning and transcriptional responses in the brain. *Pharmacol Biochem Behav.* 2012; 101:174–180. [PubMed: 22234290]
- Yamaguchi T, Kuraishi Y, Minami M, Nakai S, Hirai Y, Satoh M. Methamphetamine-induced expression of interleukin-1 beta mRNA in the rat hypothalamus. *Neurosci Lett.* 1991; 128:90–92. [PubMed: 1922954]
- 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]

Fig. 1.

Dose- and time-dependent effects of methamphetamine (METH) on core body temperature. Male, Swiss Webster mice ($n=6-8/\text{group}$) were treated with either saline or METH (25, 35, or 45 mg/kg, i.p.) and their body temperatures were recorded at various time points (15, 30, or 45 min) thereafter. METH caused a significant dose- and time-dependent response on core body temperature. Data are reported as mean $\pm$ S.E.M., *** $P<0.001$ vs. saline, post-hoc Dunnett's tests.

Fig. 2.

Dose dependent effects of methamphetamine (METH) on IL-1 β mRNA in the mouse hypothalamus, striatum, and cortex. Male, Swiss Webster mice ($n=6-8$ /group) were treated with either saline or METH (25, 35, or 45 mg/kg, i.p.) and brains were removed 45 min later. (a) Effects of METH on IL-1 β mRNA in the hypothalamus. (b) Effects of METH on IL-1 β mRNA in the striatum. (c) Effects of METH on IL-1 β mRNA in the cortex. Data are reported as mean $\pm$ S.E.M., * $P<0.05$, ** $P<0.01$ vs. saline (0 mg/kg METH), post-hoc Dunnett's tests.

Fig. 3. Correlation between core body temperature and IL-1 β mRNA expression in the hypothalamus. Male, Swiss Webster mice (n=6–8/group) were treated with saline or methamphetamine (METH 25, 35, or 45 mg/kg, i.p.) and their body temperatures were recorded after 45 min. Pearson's correlation analysis indicated no significant relationship.

Fig. 4. Effects of AZ66 and SN79 on methamphetamine (METH)-induced hyperthermia. Male, Swiss Webster mice ($n=6-8/\text{group}$) were pretreated with either saline, AZ66 (10 mg/kg, i.p.) or SN79 (3 mg/kg, i.p.). The mice were challenged 15 min later with saline or METH (35 mg/kg, i.p.) and their body temperatures were recorded at various time points (15, 30, and 45 min). (a) AZ66 pretreatment attenuated METH-induced hyperthermia. (b) SN79 pretreatment attenuated METH-induced hyperthermia. Data are reported as mean $\pm$ S.E.M., * $P<0.05$, ** $P<0.01$, *** $P<0.001$ vs. saline, # $P<0.05$, ### $P<0.001$ vs. METH, post-hoc Tukey's tests.

Fig. 5. Effects of AZ66 (AZ) and SN79 (SN) on methamphetamine (METH)-induced increases in IL-1 β mRNA in the hypothalamus. Male, Swiss Webster mice ($n=6-8$ /group) were pretreated with either saline (Sal), AZ66 (10 mg/kg, i.p.) or SN79 (3 mg/kg, i.p.). The mice were challenged 15 min later with saline or METH (35 mg/kg, i.p.) and brains were removed 45 min later. (a) AZ66 pretreatment potentiated METH-induced increases in IL-1 β mRNA in the mouse hypothalamus. (b) SN79 pretreatment potentiated METH-induced increases in IL-1 β mRNA in the mouse hypothalamus. Data are reported as mean $\pm$ S.E.M., * $P<0.05$, *** $P<0.001$ vs. saline, # $P<0.05$, ### $P<0.001$ vs. METH, post-hoc Tukey's tests.