Methamphetamine Neurotoxicity in Dopamine Nerve Endings of the Striatum Is Associated with Microglial Activation

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Received May 4, 2004; accepted May 25, 2004

ABSTRACT

Methamphetamine intoxication causes long-lasting damage to dopamine nerve endings in the striatum. The mechanisms underlying this neurotoxicity are not known but oxidative stress has been implicated. Microglia are the major antigen-presenting cells in brain and when activated, they secrete an array of factors that cause neuronal damage. Surprisingly, very little work has been directed at the study of microglial activation as part of the methamphetamine neurotoxic cascade. We report here that methamphetamine activates microglia in a dose-related manner and along a time course that is coincident with dopamine nerve ending damage. Prevention of methamphetamine toxicity by maintaining treated mice at low ambient temperature prevents drug-induced microglial activation. MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydro-1H-82958 [(±)-6-chloro-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrobromide], D2 (quiniprole), or mixed D1/D2 receptor agonists (apomorphine) do not mimic the effect of methamphetamine on microglia. Hyperthermia, a prominent and dangerous clinical response to methamphetamine intoxication, was also ruled out as the cause of microglial activation. Together, these data suggest that microglial activation represents an early step in methamphetamine-induced neurotoxicity. Other neurochemical effects resulting from methamphetamine-induced overflow of DA into the synapse, but which are not neurotoxic, do not play a role in this response.

Methamphetamine is a powerful stimulant drug of abuse. Current patterns of its use give no indication that the problem is abating. In fact, the UN Office on Drugs and Crime reported recently that abuse of amphetamines, including designer drugs such as methamphetamine and 3,4-methylenedioxymethamphetamine, now exceeds that of cocaine and heroin on a global scale (http://www.unodc.org/unodc/en/global_illicit_drug_trends.html). Complicating the medico-legal difficulties associated with any abused substance is the fact that methamphetamine causes persistent reductions of tyrosine hydroxylase activity (Haughey et al., 1999), and reductions in the dopamine transporter (Metzger et al., 2000) and humans (Volkow et al., 2001). Methamphetamine neurotoxicity has been under intense study for more than 20 years, yet much remains to be learned about how this dangerous drug causes damage to DA nerve endings. Leading theories of its neurotoxic mechanisms revolve around oxidative stress. Drug-induced oxidative stress is an attractive construct that can account for many of the effects of methamphetamine on the DA nerve ending such as inhibition of tyrosine hydroxylase activity (Haughey et al., 1999), and reductions in the dopamine transporter (Metzger et al., 2000) and the vesicle monoamine transporter (Baucum et al., 2004). It could even be an early event that leads eventually to methamphetamine-induced apoptosis (Cadet et al., 2003).

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This research was supported by National Institutes of Health Grants DA10756 and DA014392 and a VA Merit Award. Preliminary aspects of this work were presented at the National Institute on Drug Abuse-sponsored USA/Spain Binational Workshop on Drug Abuse and Addiction Research (October 2003) and the annual meeting of the Society for Neuroscience (November 2003).

Article, publication date, and citation information can be found at http://jpet.aspetjournals.org. doi:10.1124/jpet.104.070961.

ABBREVIATIONS: DA, dopamine; WIN 35,428, (-)-2-b-carbomethoxy-3-b-(4-fluorophenyl)tropane 1,5-naphthalenedisulfonate; SKF 82958, (-)-6-chloro-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrobromide; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydro-1H-82958 [(±)-6-chloro-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrobromide]; D2 (quiniprole), or mixed D1/D2 receptor agonists (apomorphine) do not mimic the effect of methamphetamine on microglia. Hyperthermia, a prominent and dangerous clinical response to methamphetamine intoxication, was also ruled out as the cause of microglial activation. Together, these data suggest that microglial activation represents an early step in methamphetamine-induced neurotoxicity. Other neurochemical effects resulting from methamphetamine-induced overflow of DA into the synapse, but which are not neurotoxic, do not play a role in this response.
However, the source of the reactive species that mediate marketed methamphetamine-induced damage is not known.

Microglia have attracted considerable attention for their roles in mediating damage to the nervous system. Immune-like in many ways (Streit, 2002), these interesting cells become activated by damage and then transmigrate to sites of injury where they can secrete an array of factors (e.g., proinflammatory cytokines, prostaglandins, nitric oxide, and superoxide) that are known to have detrimental effects on neurons (Hansich, 2002). Surprisingly, a role for microglia in methamphetamine-induced damage to the DA system has received little attention. Bowyer et al. (1994) first noted that methamphetamine resulted in activation of microglia in striatum of treated rats. These investigators concluded that microglia were increased in response to nerve ending damage and were not apparently a cause of it (Bowyer et al., 1994). We recently completed an in-depth microarray analysis of the effects of methamphetamine on striatal gene expression (Thomas et al., 2004). Numerous genes linked to microglia were activated significantly within hours of methamphetamine intoxication, suggesting the possibility that microglial activation occurs earlier in the methamphetamine toxic cascade than previously appreciated (Thomas et al., 2004).

The present studies are a direct outgrowth of our initial microarray analysis of methamphetamine and report the pharmacological characterization of microglial activation by methamphetamine in striatum, an area dense in DA nerve endings and one known to be targeted for damage by this drug. Microglial activation coincides with the onset of methamphetamine-induced damage in striatum and the extent of this effect is related to the degree of damage to DA nerve endings. Numerous nontoxic effects exerted by methamphetamine, such as inhibition of the DAT, increases in synaptic levels of DA, activation of D1 and/or D2 DA receptors, and hyperthermia, cannot explain methamphetamine-induced microglial activation. These experiments represent a logical first step in assessing the role of microglia in methamphetamine-induced nerve ending damage and suggest that microglia could be the source of numerous reactive species that are known to participate in methamphetamine toxicity.

Materials and Methods

Materials. (+)-Methamphetamine hydrochloride, cocaine hydrochloride, WIN 35,428, nomifensine, SPK 82958, quinpirole, apomorphine, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), (-)-DOI hydrochloride, pentobarbital, horseradish peroxidase (HRP)-conjugated isocitron B4 (from Gripponia simplicifolia), 3,3’-diaminobenzidine (DAB), parafomaldehyde, Triton X-100, dopamine, methanol, EDTA, all buffers, and HPLC reagents were purchased from Sigma-Aldrich (St. Louis, MO). Polyclonal antibodies against Mac-1 were obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA), and a monoclonal antibody against glial fibrillary acidic protein (GFAP) was obtained from BD Biosciences (San Diego, CA). CitriSolv and Permound were products of Fisher Scientific (Pittsburgh, PA).

Animals. Female C57BL/6 mice (Harlan, Indianapolis, IN) weighing 20 to 25 g at the time of experimentation were housed five per cage in small shoe box cages in a light- and temperature-controlled room. Mice had free access to food and water. The Institutional Care and Use Committee of Wayne State University approved the animal care and experimental procedures. All procedures were also in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Pharmacological and Physiological Procedures. Methamphetamine was injected i.p. in four injections with a 2-h interval between each injection. The dose of methamphetamine was generally 5 mg/kg injection but was varied from 1 to 10 mg/kg in some experiments (see below). Controls received i.p. injections of physiological saline on the same schedule as methamphetamine. Injection volumes were 0.1 ml/10 g body weight. Mice were also treated i.p. with various DA-reactive drugs using the same four-time injection protocol, including a 2-h interval between injections. Test drugs included MPTP (DA neurotoxin, 20 mg/kg), cocaine (psychostimulant and DAT inhibitor, 10 mg/kg), WIN 35,428 (cocaine analog and DAT inhibitor, 10 mg/kg), nomifensine (DAT inhibitor, 10 mg/kg), SKF 82958 (DA D1 receptor agonist, 10 mg/kg), quinpirole (DA D2 receptor agonist, 10 mg/kg), and apomorphine (mixed DA D1/D2 receptor agonist, 4 mg/kg). With the exception of MPTP, none of these drugs is known to be neurotoxic in the striatum, and each was used to mimic various elements of methamphetamine pharmacology, especially increased levels of synaptic dopamine and consequent pre- and postsynaptic DA receptor activation. In some experiments, core body temperature of mice was increased to simulate the hyperthermia component of methamphetamine. Two methods were used. First, mice were injected with DOI (1 mg/kg i.p., four times, 2-h interval between injections), a 5-hydroxytryptamine (serotonin) 2A (5-HT2A) receptor agonist that also causes hyperthermia (Zethof et al., 1995). Second, a heating blanket was wrapped around a small shoe box cage containing five mice, and a heat lamp was placed above the cage to increase body temperature to 40°C. Core temperatures were then maintained between 38 and 40°C over a span of 6 h to simulate body temperature changes associated with a neurotoxic regimen of methamphetamine. Body temperature was monitored throughout these treatments by telemetry using IPPT-200 implantable temperature transponders from Bio Medic Data Systems, Inc. (Seaford, DE). Core body temperatures were recorded every 20 min noninvasively using the DAS-5001 console system from Bio Medic. Finally, mice were maintained at low ambient temperature (10–12°C) starting 1 h before the onset of methamphetamine treatment and continuing for 1 h after the last drug injection (8 h in total). Low ambient temperature is a classical method used to prevent methamphetamine-induced toxicity (Ali et al., 1994; Bowyer et al., 1994; Miller and O’Callaghan, 1994). Mice were sacrificed 48 h after the above-mentioned treatments for neurochemical analyses.

Lectin Histochemical Staining and Stereology. Microglial activation was assessed by staining fixed brain sections with HRP-conjugated isocitron B4 (ILB4) as developed by Streit (1992). ILB4 stains microglia selectively in the central nervous system and has been used extensively to assess microglial activation (Ayoub and Salm, 2003; Depino et al., 2003; Lehnardt et al., 2003). At the appropriate times after treatment (specified below) mice were deeply anesthetized with pentobarbital (120 mg/kg) and perfused transcardially with ice-cold 4% paraformaldehyde. Brains were removed and stored overnight in fixative at 4°C. Sections of 50 μm in thickness were cut through the striatum (+1.2 through −0.1 mm with respect to bregma) or the area of the substantia nigra (−2.9 through −3.4 mm with respect to bregma). Sections were floated into phosphate-buffered saline (PBS) containing 0.3% H2O2 for 30 min, washed once in PBS + 0.1% Triton X-100, and then incubated in fresh PBS + 0.1% Triton X-100 for an additional 30 min. Microglia were labeled with HRP-conjugated ILB4 (10 μg/ml in 0.1% Triton X-100) overnight at 4°C. Excess ILB4 was removed by three washes with 0.1% PBS + Triton X-100 (5 min each) followed by a single wash in PBS before exposure to DAB substrate (0.1 mg/ml) in PBS for 25 min. After a single wash in PBS, sections were transferred to glass slides, air-dried, and dehydrated through a series of graded ethanol washes. Sections were incubated in CitriSolv for 5 min and then coverslipped under Permound. For all pharmacological studies presented below, brain sections from test drug-treated mice were processed simultaneously with controls and methamphetamine-treated mice to normalize staining among treatment groups. Microglial reactivity was...
viewed under the light microscope, and the number of stained cells observed after various treatments was quantified by stereological analysis using NIH Image. Cell counts were sampled from a 1.2-mm² area of the striatum by a person blinded to the treatment conditions. Cells were counted from three independent sections from all like-treated mice, bilaterally, generating an average count for each treated subject. In some experiments, sections were stained with an alternative microglial marker (Mac-1) or the astrocyte-specific marker GFAP.

**DA Tissue Content.** Depletion of striatal DA after methamphetamine treatment is widely used as an index of methamphetamine-induced toxicity to DA nerve endings. DA depletion from striatum faithfully reflects other measures of DA nerve ending damage caused by methamphetamine, such as reduced tyrosine hydroxylase immunoreactivity or reduced ligand binding to the DAT. Striata were dissected from brain 48 h after methamphetamine treatment and stored frozen at −80°C. Tissues were weighed and sonicated in 5 volumes of 0.1 perchloric acid at 4°C. Insoluble protein was removed by centrifugation, and catecholamines were adsorbed to alumina using standard protocols. After elution from alumina, the amount of DA in samples was determined by HPLC with electrochemical detection by comparison with a standard curve of authentic DA. Values for DA are reported as nanograms per milligram of tissue (wet weight) and are corrected for recovery.

**Data Analysis.** Dose-response effects of methamphetamine on striatal DA content and microglial counts were tested for significance by ANOVA. Individual treatment groups were compared with appropriate controls for DA and microglial counts with the Dunnett’s multiple comparison test in GraphPad Prism 4. Differences were considered significant if \( p < 0.05 \).

**Results**

The effects of a neurotoxic regimen of methamphetamine on striatal microglial staining are presented in Fig. 1. Striata were sampled for analysis after each of the 4 methamphetamine injections (2, 4, 6, or 8 h) and at 24, 48, 72, or 168 h after the final treatment. It can be seen in Fig. 1A that microglial staining was increased maximally at the 24 and 48 h time points. The overall effect of methamphetamine on microglial staining was significant \( (p < 0.01, \text{ANOVA}) \). The changes in microglia were significantly different from control at 24, 48, and 72 h after drug treatment. Microglial counts returned to control levels after 7 days. Figure 1B shows a striatal section from a control mouse, and Fig. 1C is a representative section from a mouse treated with methamphetamine 48 h before analysis. It is evident that the effect of methamphetamine is generalized throughout the striatum. A higher magnification of stained microglia from a methamphetamine-treated mouse is presented in Fig. 1D, showing the typical morphology of activated microglia as described previously (Ayoub and Salm, 2003; Depino et al., 2003; Lehmann et al., 2003). Examination of the entire striatum from methamphetamine-treated mice under low-power magnification indicated that microglial staining was most dense in lateral areas (data not shown), in keeping with the identical intrastratil pattern of DA damage caused by methamphetamine (Hirata et al., 1996; Harvey et al., 2000a). If microglial staining was carried out with Mac-1 antibodies in place of ILB₄, the results were the same (data not shown).

The dose of methamphetamine was varied from 1 to 10 mg/kg (for each of the four injections), and the effect on striatal dopamine and microglial activation was assessed 48 h after the last methamphetamine injection. Figure 2 shows that methamphetamine has a profound depleting effect on DA, well in keeping with previous studies (Gibb et al., 1997). The overall effect of methamphetamine dose on striatal DA was significant \( (p < 0.01; \text{ANOVA}) \). Doses of 1 to 2 mg/kg did not significantly alter striatal DA, but the higher doses of 5 to 10 mg/kg/injection for the four injections were both significantly different from control. The highest doses of 5 and 10 mg/kg caused the greatest reductions in DA to 33 and 12% of control, respectively. The effect of variation in methamphetamine dose was also reflected in microglial counts. Figure 2 shows the inverse relationship between these two measures, with higher microglial counts reflecting greater depletions of DA. The overall effect of methamphetamine...
The combined pharmacological properties of methamphetamine result in a significant increase in the synaptic levels of DA. This drug-induced release of neurotransmitter exposes synaptic D1 and D2 receptors to DA, with attendant increases in DA receptor-mediated effects. In addition, methamphetamine causes reversal of the DAT, another property that would enhance the synaptic levels of DA. Methamphetamine intoxication also causes hyperthermia, a dangerous and clinically significant side effect of abuse of this drug in humans. In an attempt to determine whether any of these effects of methamphetamine were mediating the microglial response seen, we tested a variety of compounds that act within the DA synapse or that cause increases in body temperature. The results of these test treatments on microglial activation are presented in Table 1. It is clear that methamphetamine and MPTP have the most profound effect on striatal microglial staining. Cocaine, WIN 35,428, and nomifensine, all of which are DAT blockers and psychostimulants, did not cause microglial activation in striatum, nor did a D1-selective agonist (SKF 82958), a D2-selective agonist (quinpirole), or a mixed D1/D2 agonist (apomorphine). Mice treated with the above-mentioned compounds displayed the intense behavioral reactions associated with these drugs (e.g., hyperactivity and stereotypy). Two different methods were used to test whether hyperthermia, per se, could contribute to striatal microglial activation. First, mice were treated with DOI, a nontoxic amphetamine derivative with selective actions at the 5-HT2A re-
TABLE 1
Effects of DA-reactive drugs and hyperthermia on striatal microglial activation, peak core temperature, and striatal DA content

Mice (n = 3–8 mice/group) were treated as indicated, and 48 h later brains were taken for lectin histochemistry and stereological analysis or DA determinations as described under Materials and Methods. Core body temperature was monitored throughout treatment by telemetry, and the peak temperature achieved is reported for all treatment conditions except low ambient temperature.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pharmacological Property</th>
<th>Dose&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Microglial Cell Count</th>
<th>Striatal DA</th>
<th>Peak Body Temp.&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>DA nerve terminal damage</td>
<td>5 mg/kg</td>
<td>4 ± 1</td>
<td>19.3 ± 0.71</td>
<td>37.8 ± 0.03</td>
</tr>
<tr>
<td>MPTP</td>
<td>DA nerve terminal and cell body damage</td>
<td>20 mg/kg</td>
<td>221 ± 11**</td>
<td>3.4 ± 0.53**</td>
<td>38.9 ± 0.09</td>
</tr>
<tr>
<td>Cocaine</td>
<td>DAT and SHTP blocker</td>
<td>10 mg/kg</td>
<td>316 ± 9**</td>
<td>5.8 ± 0.62**</td>
<td>37.5 ± 0.10</td>
</tr>
<tr>
<td>WIN 35,428</td>
<td>DAT blocker, cocaine analog</td>
<td>10 mg/kg</td>
<td>14 ± 3</td>
<td>17.8 ± 0.92</td>
<td>37.9 ± 0.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 ± 0.6</td>
<td>19.2 ± 0.41</td>
<td>38.9 ± 0.25</td>
<td></td>
</tr>
<tr>
<td>Nomifensine</td>
<td>DAT blocker</td>
<td>10 mg/kg</td>
<td>3 ± 0.5</td>
<td>16.7 ± 0.69</td>
<td>38.5 ± 0.27</td>
</tr>
<tr>
<td>SKF 82958</td>
<td>DA D1 receptor agonist</td>
<td>10 mg/kg</td>
<td>5 ± 0.6</td>
<td>16.4 ± 0.40</td>
<td>37.2 ± 0.25</td>
</tr>
<tr>
<td>Quinpirole</td>
<td>DA D2 receptor agonist</td>
<td>10 mg/kg</td>
<td>7 ± 1.3</td>
<td>16.8 ± 0.64</td>
<td>38.3 ± 0.26</td>
</tr>
<tr>
<td>Apomorphine</td>
<td>Mixed D1/D2 agonist</td>
<td>4 mg/kg</td>
<td>5 ± 1.3</td>
<td>19.9 ± 0.40</td>
<td>38.2 ± 0.10</td>
</tr>
<tr>
<td>DOI</td>
<td>5-HT&lt;sub&gt;2&lt;/sub&gt; agonist and hyperthermia</td>
<td>1 mg/kg</td>
<td>3 ± 0.4</td>
<td>19.1 ± 0.40</td>
<td>38.5 ± 0.23</td>
</tr>
<tr>
<td>Elevated ambient temperature</td>
<td>Hyperthermia</td>
<td>38–40°C, 6 h sustained</td>
<td>18 ± 1.3</td>
<td>15.1 ± 1.2**</td>
<td>40.0 ± 0.20</td>
</tr>
<tr>
<td>Low ambient temperature</td>
<td>Prevents methamphetamine toxicity</td>
<td>10–12°C, 8 h sustained</td>
<td>2 ± 0.5</td>
<td>18.5 ± 0.85</td>
<td>Not recorded</td>
</tr>
</tbody>
</table>

<sup>a</sup> Doses of all compounds intended to mimic indicated pharmacological properties of methamphetamine were selected from the published literature and were purposely chosen from among maximal doses for the desired effect. All injections were given on the treatment schedule used for methamphetamine (four i.p. injections of the indicated dose with 2-h intervals between injections).

<sup>b</sup> Data are presented as peak body temperature and indicate highest single body temperature measured during the treatment period, according to the method reported by Bowyer et al. (1994).

Discussion

The present experiments were designed to test the hypothesis that methamphetamine-induced toxicity to DA nerve terminals is associated with microglial activation. ILB<sub>4</sub> stains microglia selectively in brain and is used frequently as a marker for microglial activation in studies of neural damage, trauma, and neurodegeneration (Kreutzberg, 1996; Pocock and Liddle, 2001; Wyss-Coray and Mucke, 2002). Methamphetamine was shown to cause a significant increase in microglial activation 24 to 48 h after treatment. The response was transient, and returned to control within 7 days. The microglial response to methamphetamine falls well within the time frame in which damage to DA nerve terminals is initiated and amplified. For example, Baucom et al. (2004) showed recently that methamphetamine causes the formation of high molecular weight complexes of the DAT. This effect is thought to be linked to drug toxicity and is maximal 24 to 48 h after methamphetamine treatment (Baucum et al., 2004).

The microglial response to methamphetamine was generalized throughout the striatum. Microglial staining was greatest in the lateral aspects of the striatum, the same pattern seen for DA depletion (Hirata et al., 1996; Harvey et al., 2000a). The effect of methamphetamine on microglia was related to dose and showed an inverse relationship with striatal DA levels. Although the methamphetamine dose of 2 mg/kg increased microglial activation significantly, its effect on striatal DA content (approximately 10% reduction) did not quite reach statistical significance. Together, the overall temporal- and dose-response relationships between methamphetamine toxicity and microglial activation suggest that microglia are contributing to DA nerve terminal damage. Striatal sections from methamphetamine-treated mice also showed characteristic increases in GFAP staining (O’Callaghan and Miller, 1994). The morphology of GFAP-stained cells was clearly different from those stained with ILB<sub>4</sub>, indicating that ILB<sub>4</sub> was not staining astrocytes.

MPTP is a powerful neurotoxin that selectively damages DA cell bodies and nerve endings. MPTP is known to cause microglial activation (Czlonkowska et al., 1996; Du et al., 2001; Wu et al., 2002), so we tested it in comparison with methamphetamine, which does not cause damage to DA cell bodies (Harvey et al., 2000b). MPTP and methamphetamine each caused extensive microglial activation in striatum. The effects of these two drugs were very different with regard to microglial activation in the area of the substantia nigra, the site of origin of DA terminals that innervate the striatum. MPTP caused extensive microglial activation in the mesencephalon, over a similar time course seen in the striatum. Methamphetamine, on the other hand, produced no evidence of microglial activation in the area of the substantia nigra.
These findings suggest that the brain regional response of microglia to methamphetamine parallels its highly selective regional toxicity.

Methamphetamine is a powerful illicit drug. Its behavioral effects and abuse potential are thought to derive, in large part, from its ability to increase the synaptic levels of DA through release and prevention of reuptake. Its synaptic effects are then transduced by pre- and postsynaptic DA receptors. Methamphetamine intoxication is also associated with hyperthermia, a dangerous and clinically significant effect often seen in human abusers. In view of the profound behavioral and physiological effects caused by methamphetamine, we considered the possibility that one of these properties, and not its neurotoxic potential, was causing microglial activation. Therefore, various drugs that mimic selected elements of methamphetamine action were tested for effects on microglia. Doses for these treatments were purposely selected from the high-end of the range for each intended effect. Moreover, none of the drugs chosen is known to cause methamphetamine-like toxicity to DA nerve terminals. Nornicotine and WIN 35,428 are relatively specific inhibitors of the DAT, but neither drug caused an increase in microglial activation. Cocaine inhibits the serotonin transporter as well as the DAT, but despite its broader spectrum of action on monoamine transporters, it too was without effect on striatal microglia. Methamphetamine-induced release of DA exposes pre- and postsynaptic DA receptors to their neurotransmitter ligand, so we tested selective DA receptor agonists for their effects. It was observed that D1, D2, or mixed D1/D2 agonists did not alter microglial staining.

Finally, we tested whether hyperthermia played a role in microglial activation resulting from methamphetamine. DOI, a nontoxic amphetamine derivative with selectivity for the 5-HT2A receptor, causes hyperthermia (Zethof et al., 1995). We confirmed the hyperthermic effect of DOI presently, but did not observe an activation of microglia. When mice were subjected to sustained hyperthermia (6 h at 38–40°C) simulating core temperature responses achieved after methamphetamine, microglial activation was not observed. The results in Table 1 confirm that a relationship between core body temperature and microglial activation does not exist. Several conclusions can be drawn from these results. First, it is clear that microglial activation is coincident with methamphetamine-induced toxicity to DA nerve terminals. Second, it does not seem that DAT inhibition, increased synaptic DA, activation of D1 and/or D2 receptors, or hyperthermia causes the microglial response seen after methamphetamine intoxication. Third, increased synaptic 5-HT action, per se, does not seem to mediate microglial activation because neither cocaine (a serotonin transporter blocker) nor DOI (a 5-HT2A receptor agonist) caused microglial activation. Although the complex behavioral effects of the present treatments on behavior were not quantified, mice were clearly displaying symptoms that are characteristic of elevated synaptic DA and stimulation of DA receptors (e.g., hyperactivity or stereotypy). These behaviors were sustained throughout the treatment period (4 injections at 2-h intervals), so it can also be concluded that the general psychostimulant effects of methamphetamine do not contribute to microglial activation per se. The intention of these experiments was not to recreate methamphetamine neurotoxicity by testing multiple combinations of individual, nontoxic components of its action. Instead, the aim was to test the most prominent effects associated with methamphetamine, at supramaximal doses, and sustained over the same extended time frame experienced by mice during methamphetamine treatment. Despite this rather extreme approach, no single drug treatment mimicked the effect of methamphetamine on striatal microglial activation or DA depletion.

LaVoie et al. (2004) reported very recently that methamphetamine causes activation of microglia. LaVoie et al. (2004) did note somewhat less prevention of microglial activation by reducing the hyperthermic effects of methamphetamine than we observe presently. Several differences in experimental approach can explain why we see greater protection. LaVoie et al. (2004) used male rats and we used female mice, so species and sex differences are contributing factors. Perhaps the greatest difference in our studies was drug dose. LaVoie et al. (2004) administered four injections of methamphetamine at a dose of 15 mg/kg/injection, whereas we used 5 mg/kg for each of the four treatments. Use of a much lower dose could explain why we see greater protection against methamphetamine-induced microglial activation.

Bowyer et al. (1994) were perhaps the first investigators to note that methamphetamine caused microglial activation. They concluded that microglial activation occurred in response to methamphetamine-induced neuronal damage and was not causing it (Bowyer et al., 1994). Because the time of this very important observation, a great deal of evidence has accumulated that implicates microglial activation in many forms of neuronal damage and disease. These possibilities have been enumerated in many excellent review articles (Kreutzberg, 1996; Gebicke-Haerter, 2001; Pocock and Liddle, 2001; Hanisch, 2002; Polazzi and Contestabile, 2002).

Our work establishes numerous, compelling links between methamphetamine neurotoxicity and microglial activation, including very early changes in microglial-associated gene expression (Thomas et al., 2004). Although our data certainly form a strong association between microglial activation and methamphetamine neurotoxicity, they do not prove that microglia actually cause the DA nerve terminal damage associated with methamphetamine. However, several associated lines of investigation offer strong support for this possibility. First, the neurotoxic HIV Tat protein interacts synergistically with methamphetamine and causes greater toxicity in mice than either agent alone (Maragos et al., 2002; Cass et al., 2003). HIV-positive individuals who abuse methamphetamine suffer greater neuronal damage than nonabusers (Langford et al., 2003). It is well known that the AIDS virus is harbored selectively in microglia in brain (Morner et al., 2003), and it is also widely accepted that infection and activation of microglial cells play major roles in HIV-induced neurotoxicity (Gras et al., 2003). Second, methamphetamine toxicity to the DA neuronal system is prevented by chronic caffeine treatment, an effect that is thought to be mediated by caffeine-induced increases in the number of adenosine receptors (Delle Donne and Sonsalla, 1994). Adenosine receptors are now known to be localized to microglia (Hammarberg et al., 2003; Tsutsumi et al., 2004) where they exert protective effects against a variety of neurotoxic and neurodegenerative conditions (Schwarzchild et al., 2003). Third, (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate (MK-801) prevents methamphetamine toxicity by thermoregulatory (Ali et al., 1994; Miller and O'Callaghan, 1994;...
O’Callaghan and Miller, 1994) as well as nonthermoregulatory mechanisms (Bowyer et al., 2001). However, MK-801 has been shown to block microglial activation (Streit et al., 1992), a response that could protect against methamphetamine toxicity. Together, these recent developments point to microglia as participants in the DA nerve terminal damage associated with methamphetamine. We are presently assessing microglial-specific neurotoxins for their response to methamphetamine.

References


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