Dopamine Receptors and the Persistent Neurovascular Dysregulation Induced by Methamphetamine Self-Administration in Rats

Sharanya M. Kousik, T. Celeste Napier, Ryan D. Ross, D. Rick Sumner, and Paul M. Carvey

Center for Compulsive Behavior and Addiction (S.M.K., T.C.N., P.M.C.), Department of Pharmacology (S.M.K., T.C.N., P.M.C.), Department of Psychiatry (T.C.N.), Department of Neurologic Sciences (P.M.C.), and Department of Anatomy and Cell Biology (R.D.R., D.R.S.), Rush University Medical Center, Chicago, Illinois

Received June 22, 2014; accepted August 28, 2014

ABSTRACT

Recently abstinent methamphetamine (Meth) abusers showed neurovascular dysregulation within the striatum. The factors that contribute to this dysregulation and the persistence of these effects are unclear. The current study addressed these knowledge gaps. First, we evaluated the brains of rats with a history of Meth self-administration following various periods of forced abstinence. Micro-computed tomography revealed a marked reduction in vessel diameter and vascular volume uniquely within the striatum between 1 and 28 days after Meth self-administration. Microvessels showed a greater impairment than larger vessels. Subsequently, we determined that dopamine (DA) D2 receptors regulated Meth-induced striatal vasoconstriction via acute noncontingent administration of Meth. These receptors likely regulated the response to striatal hypoxia, as hypoxia inducible factor 1α was elevated. Acute Meth exposure also increased striatal levels of endothelin receptor A and decreased neuronal nitric oxide synthase. Collectively, the data provide novel evidence that Meth-induced striatal neurovascular dysregulation involves DA receptor signaling that results in vasoconstriction via endothelin receptor A and nitric oxide signaling. As these effects can lead to hypoxia and trigger neuronal damage, these findings provide a mechanistic explanation for the selective striatal toxicity observed in the brains of Meth-abusing humans.

Introduction

Methamphetamine (Meth) is a potent psychostimulant that blocks the presynaptic and vesicular uptake of biogenic amines, resulting in increased synaptic levels of these neurotransmitters (Rothman et al., 2000). Increases in synaptic dopamine (DA) may contribute to the neuropathology associated with Meth (Davidson et al., 2001), which is particularly evident in the DA-rich striatum (Thrash et al., 2010). Identifying factors that mediate Meth-induced pathology is crucial for developing therapies targeted at impeding the consequences of Meth abuse.

Self-administration by laboratory animals is a highly translatable model of human drug-taking. We previously demonstrated that Meth self-administration in rats resulted in persistent hypoperfusion that was selective to the striatum (Kousik et al., 2011), suggesting a role for Meth-induced vasoconstriction in humans. Indeed, human Meth abusers abstinent for an average of 2 years show reductions in regional cerebral blood flow (rCBF) to the striatum, with cortical regions showing less neurovascular impairment (Alhassoon et al., 2001; Chang et al., 2002; Hwang et al., 2006; Chung et al., 2010). Moreover, Meth exposure worsens tissue damage associated with ischemia and reperfusion injury in both humans and laboratory rats (Ho et al., 2009; Wang et al., 2011). Unfortunately, little is known about this phenomenon.

The striatal selectivity of Meth-induced neurovascular dysregulation, both in human Meth abusers (Chung et al., 2010) and in laboratory rats (Kousik et al., 2011), is striking. This suggests that Meth alters localized blood flow through regionally unique neurovascular units (NVUs), which contain neurons, glia, astrocytes, and vascular endothelial cells (Koehler et al., 2009; Lecrux and Hamel, 2011). Cells within the NVU interact via “neurovascular coupling” to alter the magnitude and location of blood flow changes (Koehler et al., 2009). Vasoregulatory factors produced within the local NVU are responsible for maintaining a balance between vasoconstriction and dilation to sustain proper rCBF. It is likely that Meth alters the local striatal NVU to tip the balance toward vasoconstriction and reduced blood flow.

This research was supported by the Kenneth Douglas Foundation; the Daniel F. and Ada L. Rice Foundation; and the Center for Compulsive Behavior and Addiction at Rush University Medical Center, Chicago, IL.

dx.doi.org/10.1124/jpet.114.217802

ABBREVIATIONS: μCT, micro-computed tomography; DA, dopamine; D1R, dopamine D1 receptor; D2R, dopamine D2 receptor; Et3R, endothelin receptor type A; FITC-LA, fluorescein isothiocyanate-labeled albumin; FR, fixed ratio; HIF1α, hypoxia inducible factor 1α; L741,626, 3-(4-(4-chlorophenyl-4-hydroxyperipinomethyl)indole; Meth, methamphetamine; NAc, nucleus accumbens; nNOS, neuronal nitric oxide synthase; NOS, nitric oxide synthase; NVU, neurovascular unit; PD-128, 3,4,4a,10b-tetrahydro-4-propyl-2H,5H-(1)benzopyran-4,3-b)-1,4-oxazin-9-ol; rCBF, regional cerebral blood flow; SCH23390, (+)-7-chloro-3-methyl-1-phenyl-1,2,4,5-tetrahydro-3-benzazepin-8-ol; TBST, Tris-buffered saline/ Tween-20; VTA, ventral tegmental area.
Meth markedly increases DA, a known vasoregulator (Krimer et al., 1997; Choi et al., 2006). Brain DA can activate DA D1 receptors (D1Rs) to produce vasodilation and D2 receptors (D2Rs) to produce vasoconstriction (Esaki et al., 2002). D1R located on nitric oxide synthase (NOS)–positive interneurons within the striatum directly influences nitric oxide release (Calabresi et al., 2007), leading to neurovascular dilation. D2R signaling also regulates NOS activity (Datta et al., 1999; Dimmelber et al., 1999) and decreases nitric oxide release via Akt signaling (Beaulieu et al., 2004). Both D1R and D2R signaling influence levels of endothelin-1 and subsequent activation of the endothelin receptor A (ETₐR), which mediates vasoconstriction (Morimoto et al., 2000). In addition to regulating factors that influence neurovascular flow, D2R signaling alters the activity of hypoxia inducible factor 1α (HIF1α), a transcription factor that responds to hypoxia (Barrey et al., 2009). It is likely then that Meth-induced increases in synaptic DA results in D2R signaling and alterations in striatal vasoregulatory balance leading to vasoconstriction and hypoxia. The current study evaluated the consequences of Meth on vasoregulatory factors ETₐR, neuronal NOS (nNOS), and HIF1α.

The persistence of reductions in striatal rCBF even after 2 years of abstinence in human Meth abusers (Chung et al., 2010) illustrates the potential for the pathology in the NVU to endure, and the current study evaluated this possibility. To emulate what occurs in human Meth abuse, we used rats that self-administered Meth, and evaluated neurovascular dysregulation during forced abstinence of various durations. We hypothesized that Meth self-administration alters vasoregulators within the striatal NVU. Outcomes supported the hypothesis and revealed that Meth-induced effects were mediated by DA receptors and persisted for almost 2 months.

Materials and Methods

Animals. Male Sprague-Dawley rats (n = 160; Harlan Laboratories, Indianapolis, IN) weighing 225–250 g upon arrival were housed in pairs, handled daily, and acclimated to environmentally controlled conditions for 1 week before experimentation. Rats were 7–8 weeks old upon arrival and sacrificed at varying time points during forced abstinence following Meth self-administrations such that the oldest rats used in this study were approximately 15 weeks of age. Rats had access to food and water ad libitum throughout the study. All procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals Eighth Edition (National Research Council, Washington, DC) with protocols approved by the Rush University Institutional Animal Care and Use Committee.

Drugs. (+)-Methamphetamine HCl (Sigma-Aldrich, St. Louis, MO) was dissolved in sterile saline. For self-administration, Meth was delivered via an infusion of 0.1 mg/kg per 0.1 mL. For acute drug treatment studies, a 2.5 mg/kg i.p. dose of Meth was administered. R(+)-SC123390 HCl (R(+)-7-chloro-3-methyl-1-phenyl-1,2,4,5-tetrahydro-3-benzazepine-8-ol hydrochloride; Sigma-Aldrich), a D1R-prefering antagonist, was dissolved in distilled water and administered at a dose of 0.1 mg/kg. This dose of SC123390 is selective for the D1R (Hyttel, 1983; Iorio et al., 1983) and blocks stereotypic behavior and increased locomotion induced by either amphetamine or apomorphine (Christensen et al., 1984; Mailman et al., 1984; Napier et al., 1986). L741,626 [3+(4-(4-chlorophenyl-4-hydroxyperidinomethyl)indole; Toecis, Minneapolis, MN], a D2R-prefering antagonist, was dissolved in 70% ethanol and administered at a dose of 1 mg/kg. This dose decreases behaviors (i.e., rotations, locomotion, yawning) induced by D2/D3R-selective agonists quinpirole and PD-128 [3,4,4a,10b-tetrahydro-4-propyl-2H,5H-1benzoazepyrano (4,3-b)-1,4-oxazin-9-ol] (Millan et al., 2000, 2004). For the acute treatment studies, all drugs were given intraperitoneally.

Self-administration Protocols. To allow intravenous catheter implantation, 64 rats were anesthetized using isoflurane, then a custom-made Silastic tubing catheter (0.3 mm i.d. × 0.64 mm o.d.; Dow Corning Co., Midland, MI) was implanted in the right jugular vein as previously described (Graves and Napier, 2011). Following surgery, catheters were flushed daily with 0.1–0.2 ml of sterile saline to maintain catheter patency. Catheter patency was determined by ease of flushing during recovery followed by consistent self-administration of Meth. Rats were given 5 days of postsurgery recovery before the first day of self-administration.

Self-administration sessions lasted for 3 h/day and were done in operant chambers (30.5 × 24.1 × 21.0 cm; Med-Associates, St. Albans, VT) equipped on one wall with two retractable levers and a stimulus light above each lever, and a single house light on the opposite wall. Chambers were enclosed in ventilated, sound-blocking cabinets. For our paradigm, the left lever was deemed as the “active” lever, whereas the right was “inactive.” Pressing the active lever resulted in infusion of 0.1 mg/kg per 0.1 ml Meth over 6 seconds, which was accompanied by illumination of the stimulus cue light above the lever. Subsequently, the house light was illuminated and remained on for 20 seconds indicating a “time-out” period. Active lever presses were recorded during this time-out period, but had no programmed consequence. Inactive lever presses never had programmed consequences. Rats were trained for 14 days to self-administer Meth; animals were not food restricted, and minimal shaping was used. Rats self-administered on a fixed-ratio 1 (FR1; 1 lever press/infusion) schedule of reinforcement for days 1–7 followed by a FR5 schedule for days 8–14 to increase behavioral responses (Acosta et al., 2008). Stable self-administration was operationally defined as less than 15% reinforcement variability between days 13 and 14; rats failing to meet this criterion were excluded from the study. Control rats underwent identical surgical procedures, but with yoked-saline, noncontingently administered according to infusion patterns of a paired, Meth, self-administering rat. Rats were sacrificed at 1, 14, 28, or 56 days following the last operant session for micro–computed tomography (μCT).

Acute Drug Administration. To verify if DAergic receptors were involved in the neurovascular alterations induced by Meth, rats (n = 16 per treatment group) were administered two injections, with 30 minutes between the first and second treatment. Accordingly, the treatment groups included the following: vehicle + saline, vehicle + Meth, SC123390 + saline, L741,626 + saline, SC123390 + Meth, and L741,626 + Meth. All rats were killed 2 hours following the final drug treatment of fluorescein isothiocyanate–labeled albumin (FITC-LA) imaging, μCT, or Western blotting.

FITC-LA Perfusion. Following acute drug treatment, rats (n = 12) were anesthetized with 400 mg/kg per milliliter chloral hydrate then intracardially perfused with FITC-LA (molecular mass = 68–70 kDa; Sigma-Aldrich) to assess neurovascular perfusion as previously described (Carvey et al., 2005).

Micro-Computed Tomography. Meth self-administering rats (n = 64) or rats treated acutely with Meth and the DA-prefering antagonists (n = 36) were anesthetized with 400 mg/kg per milliliter chloral hydrate and intracardially perfused with Microfil-MV (FlowTech Inc., Carver, MA), a radiopaque agent used for μCT. Microfil-MV perfusions were done as described by Langheinrich et al. (2010). All samples were scanned in a μCT manufactured and developed by SCANCO Medical (SCANCO μCT 50; Brüttisellen, Switzerland). The X-ray system used the following scan parameters: 45 kV, 200 mA, and 9 W with 10-μm isotropic voxels and a 500-millisecond integration time. Segmentation was performed to separate perfused vessels from the surrounding soft tissue using the manufacturer’s software, and the mean vessel diameter (millimeters) and vascular volume fraction were calculated. A 1.5-mm scanning width was used to evaluate any Meth-induced neurovascular changes within the striatum, primary motor cortex, nucleus accumbens (NAc), substantia nigra, ventral tegmental area (VTA), and parietal association cortex.

Western Blots. Following acute drug treatment, rats (n = 48) were killed via rapid decapitation, and brains were removed in less
than 45 seconds and cooled rapidly in ice-cold saline. The bilateral rostral striatum and substantia nigra were dissected and fast-frozen on dry ice. Tissue samples were resuspended in lysis buffer [25 mM 2-14-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid, pH 7.4, 500 mM NaCl, 2 mM ethylenediaminetetraacetic acid, 1 mM dithiotreitol, 1 mM phenylmethylsulfonyl fluoride, 20 mM NaF, 1× phosphatase inhibitor cocktails (Sigma-Aldrich), 1× protease inhibitor cocktail (Calbiochem, La Jolla, CA), and 0.1% Nonidet P-40]. Samples were then sonicated for 5 seconds, centrifuged for 2 minutes, aliquoted, and stored at −80°C until assayed. Samples from the rostral striatum and substantia nigra (20 μg for ETAR, and 40 μg for HIF1α and nNOS) were loaded and electrophoresed on 4–20% gradient Tris-HCl gels (Bio-Rad, Hercules, CA) and then transferred to polyvinylidene difluoride membranes for Western blotting. Nonspecific binding sites were blocked using 5% nonfat dry milk in Tris-buffered saline/Tween-20 (TBST; 20 mM Tris, 137 mM NaCl, 0.5% Tween-20, pH 7.4) for 1 hour at room temperature. Membranes were incubated in primary antibodies for ETAR (1:1000; Abcam, Cambridge, MA), nNOS (1:500; Cell Signaling Technology, Danvers, MA), or HIF1α (1:500; Abcam) at 4°C overnight. After washing in TBST, membranes were incubated in goat antirabbit secondary antibody (Millipore, Billerica, MA) for 1 hour at room temperature. Membranes were again washed in TBST, immersed in enhanced chemiluminescent substrate (SuperSignal West Pico; Pierce Biotechnology, Rockford, IL) for 5 minutes, and exposed to HyBlot CL film (Denville Scientific, Metuchen, NJ). Membranes were then stripped in buffer (62.5 mM Tris, 2% sodium dodeyl sulfate, 100 mM β-mercaptoethanol, pH 6.8) for 30 minutes at 60°C and reprobed for actin (1:10,000; Santa Cruz Biotechnology, Santa Cruz, CA), which was used as a loading control for ETAR, nNOS, and HIF1α. Densities of the immunoreactive bands were analyzed using ImageJ software (NIH, Bethesda, MD).

Statistics. Data are presented as means and S.E.M. For μCT analysis after Meth self-administration, a mixed-model analysis of variance with post-hoc independent samples t tests (Bell and Rowley, 2011) were used to analyze the changes in outcome measures associated with Meth self-administration compared with saline-yoked controls (i.e., Treatment) over time (i.e., Time). Thus, Meth self-administering rats were compared with saline-yoked rats such that each group was only used for one t test. μCT and Western blotting data obtained from acute drug treatment groups were analyzed using a one-way analysis of variance. When appropriate, a Newman-Keuls post-hoc analysis was performed. For all analyses, α = 0.05. Data were analyzed using SPSS Statistics (IBM, Somers, NY).

Results

Methamphetamine Self-Administration. Sixty-four rats were trained in self-administration. At 1, 14, 28, or 56 days after the last operant session, brain tissue was harvested from eight Meth self-administering rats and eight saline-yoked controls for μCT. The average intake of Meth for the last three sessions of self-administration was 3.71 ± 0.25 mg/kg/day. Daily averages for active lever presses, inactive lever presses, and infusions for Meth self-administering rats over the 14-day period are shown in Fig. 1. Increases in active lever pressing for Meth self-infusion during days 8–14 when rats were trained on an FR5 schedule of reinforcement demonstrated that the rats learned to increase the behavioral response (i.e., lever pressing) to maintain their desired Meth infusions.

Neurovascular Alterations after Methamphetamine Self-Administration. μCT analysis was evaluated in the striatum, primary motor cortex, NAc, substantia nigra, VTA, and parietal association cortex. The striatum was assessed in the present study as our prior work showed hypoperfusion uniquely in this brain region after Meth self-administration using the same protocol used here (Kousik et al., 2011). We also assessed vascular alterations in the substantia nigra, the region of origin for striatal afferents, and primary motor cortex, which receives inputs from the striatum. The NAc, VTA, and parietal association cortex served as controls, as these regions do not show Meth-induced neurovascular dysregulation in human Meth abusers (Chung et al., 2010). Figure 2A shows a representative μCT reconstruction of the brain section in which vascular alterations were assessed for the striatum, primary motor cortex, and NAc. Figure 2B shows a representative μCT reconstruction of the brain section in which vascular alterations were assessed for the substantia nigra, VTA, and parietal association cortex.

In the striatum (Fig. 3A), there was an approximately 50% decrease in mean vessel diameter at 1 day (P < 0.001) and 14 days (P < 0.001) after Meth self-administration when compared with saline-yoked controls at each withdrawal time (Treatment F(1,14) = 62.18, P < 0.001; Time F(3,42) = 7.78, P < 0.001; interaction of Treatment and Time F(3,42) = 4.10, P = 0.01). There was also a 30% decrease in the vascular volume fraction at 1 day (P < 0.01) and 14 days (P < 0.01) as well as a 12% decrease at 28 days (P < 0.05) following Meth self-administration when compared with saline-yoked controls at each withdrawal time (Treatment F(1,14) = 16.67, P = 0.001; Time F(3,42) = 10.71, P < 0.001; interaction of Treatment and Time F(3,42) = 2.83, P = 0.05). A frequency distribution assessment of vessel diameter alterations in the striatum (Fig. 3B) revealed that the striatal microvessels (10–30 μm) were most sensitive to Meth-induced neurovascular changes, whereas larger vessels remained largely unaffected.

In the primary motor cortex (Fig. 3C), there was a 25% decrease in mean vessel diameter at 1 day following Meth self-administration when compared with saline-yoked controls for...
that day (there were no effects of Treatment, Time, or the interaction of Treatment and Time). There were no changes in vascular volume within the primary motor cortex after Meth self-administration when compared with saline-yoked controls or over the total time of forced abstinence.

In the substantia nigra (Fig. 3D), there were no differences in mean vessel diameter or vascular volume between Meth self-administering rats and saline-yoked rats or over the total time of forced abstinence. Similarly, no neurovascular alterations were detected in the NAc, VTA, or parietal association cortex (data not shown). Taken together, these data revealed that Meth-induced neurovascular dysregulation persists even after the drug is no longer in the system, and that these effects are selective to the striatum.

Neurovascular Alterations after Acute Methamphetamine Treatment. Fluorescence imaging of FITC-LA allowed for visualization of the neurovasculature (Fig. 4). To evaluate the potential role of DA receptors in Meth-induced vasoconstriction within the striatum, rats were treated with either a D1R-prefering antagonist, SCH23390 (0.1 mg/kg i.p.), or D2R-prefering antagonist, L741,626 (1 mg/kg i.p.), prior to 2.5 mg/kg i.p. Meth. Saline controls (vehicle + saline) showed normal vascular patterns in the striatum and in the overlying cortex, whereas acute Meth treatment (vehicle + Meth) resulted in an overt absence of FITC-LA in the striatum with normal FITC-LA perfusion in the overlying cortex, similar to the perfusion patterns we reported previously (Kousik et al., 2011). Pretreatment with SCH23390 (D1R-prefering antagonist) did not affect Meth-induced hypoperfusion, whereas pretreatment with L741,626 (D2R-prefering antagonist) resulted in a normal FITC-LA perfusion pattern in the striatum. As Meth also increases synaptic serotonin levels, we tested the ability of methysergide, a nonselective serotonin receptor antagonist, to alter Meth-induced effects. Methysergide (1 mg/kg i.p.) pretreatment did not alter Meth-induced hypoperfusion (data not shown).

In the striatum, acute Meth (vehicle + Meth) decreased the mean vessel diameter by 38% \( F_{(1,35)} = 4.89, P < 0.05 \) when compared with saline controls (vehicle + saline; Fig. 5). Pretreatment with SCH23390 did not alter Meth-induced reductions in vessel diameter, whereas pretreatment with L741,626 attenuated the Meth-induced reductions. Acute Meth also decreased the striatal vascular volume fraction \( F_{(3,35)} = 11.67, P < 0.01 \) by 43% when compared with saline controls (Fig. 5). Pretreatment with either SCH23390 or L741,626 pretreatment increased the volume fraction above that measured for saline controls. Treatment with either SCH23390 or L741,626 alone did not have an effect on the striatal vasculature.

Vasoregulatory Factors Involved in the Neurovascular Alterations Induced by Methamphetamine. Vascular tone is a consequence of a balance of vasoregulators. It is likely that Meth tips this balance toward constriction in the striatum. Using the acute drug treatment paradigm described earlier, Western blots in the rostral striatum and substantia nigra were conducted to identify vasoregulatory factors involved in Meth-induced neurovascular dysregulation that are regulated by DA receptors (Fig. 6). ET\(_{\mathrm{A}}\)R on smooth muscle cells...
surrounding the vascular endothelial cells is responsible for endothelin-1 vasoconstriction. Western blotting revealed that acute Meth increased levels of ETAR \[ F_{(5, 47)} = 10.16, P < 0.001 \]. Pretreatment with either SCH23390 or L741,626 attenuated this increase and returned levels back to that of saline controls. nNOS catalyzes the production of nitric oxide, a major vasodilator. Western blotting revealed that acute Meth decreased nNOS \[ F_{(5, 47)} = 4.24, P < 0.01 \], and pretreatment with either SCH23390 or L741,626 attenuated this decrease and returned levels back to that of saline controls. As the FITC-LA imaging and \( \mu \)CT data showed that D2R are at least partially responsible for Meth-induced neurovascular dysregulation, we evaluated the influence of D2R signaling on HIF1\( \alpha \), a transcription factor that responds to hypoxia. Acute Meth increased levels of HIF1\( \alpha \) \[ F_{(5, 47)} = 7.97, P < 0.01 \]. Pretreatment with SCH23390 had no effect, whereas pretreatment with L741,626 attenuated Meth-induced increases in HIF1\( \alpha \). Treatment with SCH23390 + saline or L741,626 + saline had no effect on any of the vasoregulatory factors. Taken together, these data suggest that alterations in the vasoregulatory factors (i.e., ET\( \alpha \)R and nNOS) that could affect vascular tone are complex and mediated by both D1R and D2R, whereas the response to hypoxia after Meth-induced vasoconstriction is at least partially regulated by D2R-activated HIF1\( \alpha \).

**Discussion**

The present study revealed the effects of Meth self-administration on the vasculature. On average, the rats self-titrated 3.6 ± 0.2 mg/kg Meth per 3-hour session for the 14 days of the task. This dose is considerably lower than what is typically experimenter-administered to assess Meth-induced neurotoxicity, i.e., 9–40 mg/kg Meth wherein high-dose acute Meth treatment can result in severe losses (e.g., 60–70%) in striatal presynaptic DA markers within 1 day after treatment (see review by Yamamoto et al., 2010). In the same Meth self-administering rats used in the present study, we reported a progressive reduction in striatal tyrosine hydroxylase with a 50% reduction at 56 days following self-administration (Kousik et al., 2014), which is reminiscent of what is reported in human imaging studies, where Meth abusers abstinent from the drug for an average of 3 years only show a 20–30% loss in presynaptic striatal DA markers (McCann et al., 1998). Outcome differences between acute Meth treatment and self-administration may reflect differences in doses as well as the differences in the consequences of noncontingent versus contingent administration (i.e., self-administration) (Jacobs et al., 2003; Palamarchouk et al., 2009; Reichel et al., 2012). For example, prior studies reported that Meth self-administration in rats resulted in higher levels of neurotensin (Frankel et al., 2011) and persistent reductions in DA and glutamate transmission in the NAc (Lominac et al., 2012) that were not evident in noncontingently treated rats. The present study evaluated the temporal pattern of vascular changes (i.e., maintenance of vascular dysfunction or...
amine, an shown), and Polesskaya et al. (2011) administered phentol- tated rats with methysergide prior to Meth and observed no result of changes in these monoamines. However, we pre-

vascular effects observed in the striatum could have been a greater concentrations of DA in the striatum. Since Meth lenticulostriate branches, perhaps due to the significantly branches of the posterior cerebral artery (Uchida et al., 2010).

or parietal association cortex, all of which are perfused by (Feekes and Cassell, 2006), or in the substantia nigra, VTA, recurrent artery of Heubner from the anterior cerebral artery alter the neurovasculature in the NAc, which is perfused by the Meth-induced vasoconstriction, whereas larger vessels (>20 μm) were not significantly altered. These microvessels encompass the arterioles within the microvasculature which are the primary site for local blood flow regulation by the NVU. Thus, constriction of these vessels exerts a critical reduction in brain O₂ and potential hindrance of toxin removal from the central nervous system. Overall, the Meth-induced reductions in vessel diameter and volume appeared to return to levels of saline-yoked controls by 56 days after Meth self-administration. However, striatal rCBF reductions leading to hypoxia prior to reperfusion may have detrimental consequences.

We also observed a decrease in vessel diameter in the primary motor cortex overlying the striatum at 1 day after Meth self-administration, although this effect was not observed at 14 days. Both the primary motor cortex and striatum are perfused by branches of the middle cerebral artery (Feekes and Cassell, 2006), the most common site of stroke (Engel et al., 2011). The lenticulostrate branches of the middle cerebral artery perfuse the striatum, which, based on the time-course data, was affected longer. In contrast, Meth self-administration did not alter the neurovasculature in the NAc, which is perfused by the recurrent artery of Heubner from the anterior cerebral artery (Feekes and Cassell, 2006), or in the substantia nigra, VTA, or parietal association cortex, all of which are perfused by branches of the posterior cerebral artery (Uchida et al., 2010).

These data suggest that Meth has its greatest effects on the lenticulostrate branches, perhaps due to the significantly greater concentrations of DA in the striatum. Since Meth increases serotonin and norepinephrine in addition to DA, the vascular effects observed in the striatum could have been a result of changes in these monoamines. However, we pre-
treated rats with methysergide prior to Meth and observed no attenuation of Meth-induced vascular changes (data not shown), and Polesskaya et al. (2011) administered phenol- amine, an α-adrenergic antagonist, prior to Meth and similarly reported no attenuation in vascular changes, suggesting that, among the three neurotransmitters affected by Meth, DA is the primary vasoregulator in the striatum. Clinical studies showed reductions in rCBF in the striatum and several cortical areas for up to 2 years following Meth abuse (Chung et al., 2010), whereas our data showed recovery of the striatal NVU by 56 days after Meth self-administration in rats. The seemingly longer neurovascular effects documented in Meth-abusing humans versus Meth self-administering rats likely reflect species differences, Meth intake, and the neurobiobehavioral factors related to human drug-taking that are not mirrored well in the laboratory.

This study also assessed the role of DA receptors and vasoregulatory factors involved in Meth-induced neurovascular pathology. In the striatum, D1Rs are located on NOS + interneurons and medium spiny neurons projecting to the internal globus pallidus and substantia nigra pars reticulata, whereas D2Rs are located on presynaptic DA terminals as well as postsynaptic cholinergic interneurons and medium spiny neurons projecting to the external globus pallidus (Calabresi et al., 2007). D2Rs are also located on vascular endothelial cells within the NVU (Volkow et al., 2011). Our data revealed that Meth-induced reductions in striatal vessel diameter were attenuated by pretreatment with L741,626 (D2R-prefering antagonist) but not by SCH23390 (D1R-prefering antagonist), suggesting that D2R activation is largely responsible for the vasoconstricting effects of Meth. As blood flow changes are due to alterations in vasoregulatory balance, it is likely then that Meth tips this balance, through D2R→D1R signaling, toward vasoconstriction.

It is possible that both D1R and D2R influence levels of ET₄R and nNOS through separate mechanisms. Our Western blot studies revealed that in the striatum, acute Meth increased ET₄R and decreased nNOS levels, leading to constrictions. Pretreatment with the D1R-prefering antagonist SCH23390 or the D2R-prefering antagonist L741,626 attenuated these changes. Thus, D1R and D2R may influence endothelin-1 production and subsequent changes in ET₄R (Morimoto et al., 2000). Interestingly, Seo and colleagues (2012) showed that in isolated intracerebral arteries of mice, Meth-induced vasoconstriction was mediated by ET₄R, independent of endothelin-1 activity. NOS activity is also regulated by both DA receptors. D1Rs located on NOS + interneurons directly influence nitric oxide release (Calabresi et al., 2007), whereas D2R-mediated signaling influences NOS activity (Beaulieu et al., 2004).
We also assessed the role of D2R-mediated HIF1α activity in response to striatal hypoxia. We showed that acute Meth increased HIF1α levels when compared with saline controls. L741,646 pretreatment reversed this increase, suggesting that D2R mediates increases in HIF1α activity, i.e., transcription of genes targeting angiogenesis, regulation of blood flow, and metabolism. Jiang et al. (2002), in response to striatal hypoxia after Meth. Thus, even though the Meth-induced changes in ET αR and nNOS are likely involved in the neurovascular effects observed, the increase in HIF1α was the only factor uniquely affected by L741,646, suggesting that the response to hypoxia following Meth-induced vasoconstriction is primarily mediated by D2R. None of the vasoregulators assessed were altered in the substantia nigra, emphasizing the importance of the local striatal NVU in regulating Meth-induced neurovascular dysregulation.

Despite the fact that the brain receives a significantly disproportionate percent of cardiac output, the function of the vasculature in neuropathologies has been grossly understudied. The current study provided novel data identifying specific vasoregulatory factors that influence the persistent neurovascular dysregulation produced by Meth. Therapeutically targeting vasoregulatory factors to improve brain perfusion may help rescue the brain from additional toxic effects of Meth, e.g., oxidative stress and excitotoxicity, and impede the neuropathological consequences of Meth abuse.

Acknowledgments
The authors thank the Rush University μCT and Histology Core wherein the μCT experiments were executed. The authors also thank Dr. Amanda Persons for exceptionally helpful insights into this project.

Authorship Contributions
Participated in research design: Kousik, Napier, Carvey. Conducted experiments: Kousik. Contributed new reagents or analytic tools: Ross, Sumner. Performed data analysis: Kousik, Ross. Wrote or contributed to the writing of the manuscript: Kousik, Napier, Ross, Sumner, Carvey.

References


Address correspondence to: Dr. Paul M. Carvey, Cohn Research Building, Suite 406, 1735 W. Harrison Street, Chicago, IL 60612. E-mail: paul_carvey@rush.edu