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High Impact Short Article

In vivo evidence of methamphetamine induced attenuation of brain tissue oxygenation as measured by EPR oximetry

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ABSTRACT

Abuse of methamphetamine (METH) is a major and significant societal problem in the US, as a number of studies have suggested that METH is associated with increased cerebrovascular events, hemorrhage or vasospasm. Although cellular and molecular mechanisms involved in METH-induced toxicity are not completely understood, changes in brain O_2 may play an important role and contribute to METH-induced neurotoxicity including dopaminergic receptor degradation. Given that O_2 is the terminal electron acceptor for many enzymes that are important in brain function, the impact of METH on brain tissue pO_2 *in vivo* remains largely uncharacterized. This study investigated striatal tissue pO_2 changes in male C57BL/6 mice (16–20 g) following METH administration using EPR oximetry, a highly sensitive modality to measure pO_2 *in vivo*, *in situ* and in real time. We demonstrate that 20 min after a single injection of METH (8 mg/kg i.v.), the striatal pO_2 was reduced to 81% of the pretreatment level and exposure to METH for 3 consecutive days further attenuated striatal pO_2 to 64%. More importantly, pO_2 did not recover fully to control levels even 24 h after administration of a single dose of METH and continual exposure to METH exacerbates the condition. We also show a reduction in cerebral blood flow associated with a decreased brain pO_2 indicating an ischemic condition. Our findings suggests that administration of METH can attenuate brain tissue pO_2, which may lead to hypoxic insult, thus a risk factor for METH-induced brain injury and the development of stroke in young adults.

Introduction

Methamphetamine (METH) abuse continues to be a significant, but not adequately addressed, societal problem in the US. While METH-induced neurotoxicity has been studied for decades, the cellular and molecular mechanisms underlying this toxicity are poorly understood. Acute and chronic METH abuse damages multiple organs; however, neurotoxicity in the brain is the more prominent. *In vitro*, *ex vivo* and clinical studies suggest that METH abuse and neurotoxicity are associated with hypoxia as a result of hypoperfusion and vasoconstriction (Kousik et al., 2011), cerebrovascular incidents, such as hemorrhagic and ischemic stroke (Ho et al., 2009; Perez et al., 1999; Wang et al., 2001) and oxidative stress (Cadet and Brannock, 1998; Riddle et al., 2006). Studies have suggested attenuation of oxygen (O_2) in the brain to be associated with uncoupling of mitochondria after METH administration (Shiba et al., 2001). Additionally, it has been shown that METH displaces vesicular dopamine and serotonin, which are oxidized to dopamine-type quinones in the striatum. Thus, METH-induced dopaminergic receptor degeneration in addition to pathways that elicit mitochondrial toxicity and increased glutamine release may play an important role in its neurotoxicity (Pubill et al., 2005; Yamamoto and Zhu, 1998).

Molecular O_2 plays a central role in the control of brain physiology, *e.g.* O_2 is the terminal acceptor for enzymes that are crucial to the biosynthesis of the neurotransmitters, dopamine, serotonin and norepinephrine. Thus, changes in tissue O_2 directly impact homeostasis and brain tissue is highly sensitive to changes in availability of O_2, in which pathological changes can occur if there are significant changes in the delivery or utilization of O_2 in the brain (Liu and Rosenberg, 2005; Love, 1999; Zauner et al., 2002). While it is well established that

Abbreviations: METH, methamphetamine; pO_2, partial pressure of oxygen; EPR, electron paramagnetic resonance; SpO_2, arterial blood gas; HR, heart rate; BR, breath rate; CBF, cerebral blood flow; LiPc, lithium phthalocyanine.

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decreased levels of O_2 in the brain occur following stroke and traumatic brain injury, only in recent years have preliminary ex vivo data surfaced to suggest that METH-neurotoxicity may be linked to changes in O_2 levels (Kousik et al., 2011; Shiba et al., 2001; Wang et al., 2001) and as a risk factor for the development of stroke in young adults (Ho et al., 2009; Perez et al., 1999). Despite these extensive results, there is no direct *in vivo in situ* and in real time evidence regarding METH-induced alterations in brain tissue partial pressure of oxygen (pO_2). It remains unclear whether METH is a predisposing factor to ischemic brain injury and, if so, whether changes in pO_2 are a significant contributing factor to METH-induced cerebral damage *in vivo*.

Surprisingly, given the significance of O_2 in brain physiology, the accurate measurement of O_2 in the brain using different modalities is not a trivial task. For instance, O_2 in biological systems has been determined by more invasive methods such as Clark-type electrodes and fluorescence quenching of a ruthenium dye and Blood oxygen level-dependent (BOLD) MRI imaging provides only quantitative estimate of O_2 (Baudelet and Gallez, 2002; Elas et al., 2003). Experimental efforts are also hampered by the physical difficulty of measuring O_2-delivery to the tissue at the microvascular level. With development of *in vivo* electron paramagnetic resonance (EPR) oximetry (Ahmad and Kuppusamy, 2010; Dunn and Swartz, 2003), we have measured cerebral interstitial pO_2 in several ischemic rodent models using the minimally invasive O_2 sensitive paramagnetic probe, lithium phthalocyanine (LiPc) (Liu et al., 1993, 1995; Shen et al., 2009). LiPc has several desirable properties as a probe for cerebral pO_2 including high sensitivity, resistance to chemical reactions, and high degree of inertness in biological systems for long- or short-term studies *in vivo, in situ* and in real time (Elas et al., 2003; Liu et al., 1995, 2004).

To further understand METH-induced effects on the brain we have investigated the local interstitial levels of pO_2 in the striatum after METH administration using the novel technique of EPR oximetry and the spin probe, LiPc. Herein, we report that METH-treated mice experience decreased local interstitial levels of pO_2 in the striatum. Additionally, the observed attenuation of brain pO_2 is accompanied by a decrease in cerebral blood flow (CBF), indicating an ischemic condition and moreover, after single non-lethal dose of METH, brain tissue pO_2 does not appear to fully recover to normal physiological levels.

Materials and methods

Animals. The Laboratory Animal Care and Use Committee of the UNM HSC approved all experimental protocols. Male C57BL/6 mice, 16–20 g, were obtained from Charles River Laboratory (Wilmington, MA, USA). Animals were maintained in a climate-controlled vivarium with a 12 h light–dark cycle and free access to food and water.

For all surgical stereotaxic LiPc implantation procedures, 4.0% isoflurane in N_2O:O_2 (70:30%) was used for anesthesia induction, and anesthesia was maintained with 1% isoflurane in mice. LiPc was a gift from Dr. Harold Swartz (NIH *In vivo* EPR Center, Dartmouth College, NH, USA). Animals were anesthetized throughout all EPR, MRI and Pulse Ox measurements with 1% isoflurane in N_2O:O_2 (70:30%) after induction at 4.0% isoflurane in N_2O:O_2 (70:30%). Physiological monitoring during all procedures comprised of measurement and maintenance of core (rectal) temperature at 37.5 ± 0.5 °C using a heating pad, a heat lamp or a warm air heater in the MRI.

Drugs and chemicals. *d*-Methamphetamine hydrochloride was purchased from Sigma (St. Louis, MO). Drugs were dissolved in 0.9% saline vehicle. Animals, under anesthesia, were given a single dose of METH (8 mg/kg i.v.) for 3 consecutive days. This dosage was selected based on previous studies in which similar single large doses of METH demonstrate persistent behavioral and neurochemical changes to assess its effects on the central nervous system of various mammalian species and to reflect METH overdoses or binging in human abusers (Cadet et al., 2003). Control mice were injected with an equal volume of 0.9% saline vehicle.

Implantation of EPR oximetry probe LiPc. For every animal, correct assignment of the implantation site in the striatum was determined using the Mouse Brain Atlas (Paxinos and Franklin, 2001) and was confirmed by MRI. Under anesthesia, a pin hole on the parietal skull was made at the stereotaxic position of AP: +0.5 mm and L: +1.5 mm with respect to bregma. A small LiPc crystal (approximate diameter 0.2 mm) was placed at a depth of −3.5 mm using a microdialysis guide cannula with an inner diameter of 0.24 mm (CMA microdialysis, Stockholm, Sweden). LiPc crystal was placed in the striatum of the right hemisphere of mice. Mice were allowed to recover from implantation 48–72 h before further study.

Measurement of cerebral pO_2 by EPR oximetry with LiPc. For non-invasive *in vivo* measurement of local cerebral pO_2 in the anesthetized mouse before and directly after injection of METH, EPR oximetry was conducted according to previously described methods (Liu et al., 1993, 1995; Shen et al., 2009) with some modification. Briefly, an external loop resonator was placed over the position where LiPc was implanted, and an EPR spectrum was recorded using a Bruker EleXsys E540 EPR spectrometer equipped with an L-band bridge (Bruker Instruments, Billerica, MA, USA). The resonator has advanced automatching and autotuning capabilities that correct for any slight animal movements. The EPR spectrum was acquired with a scan time of 40 s, and 5 scans were obtained and averaged to produce significant signal-to-noise ratio to allow accurate fitting. The peak-to-peak line width of the spectrum was obtained via computer line-fitting, and converted to pO_2 values according to a calibration curve for LiPc as previously described (Liu et al., 1995; Liu et al., 2004; Shen et al., 2009). EPR acquisition parameters: microwave power of 18 mW, a microwave frequency of 1.07 GHz, a center magnetic field strength of 380 G, a scan range of 1.0 G, with a modulation amplitude of less than one-third of the intrinsic EPR line-width.

Confirmation of LiPc implants and measurement of CBF by MRI. Mice with LiPc implants were placed in a custom-built holder and moved to the isocenter of the magnet before obtaining MRI images. Throughout the imaging session, animals were anesthetized and monitored in real time. MR imaging was performed on a 4.7 T Biospec® dedicated research MR scanner (Bruker Biospin, Billerica, MA), equipped with 500 mT/m (rise time 80–120 μs) gradient set (for performing small animal imaging) and a small bore linear RF coil (ID 72 mm). LiPc implant position was confirmed using T2-weighted 2D RARE (rapid acquisition with relaxation enhancement) imaging using the following parameters: TR/TE, 4000/65 ms; FOV, 2.5 cm ± 2.5 cm; slice thickness, 1.0 mm; slice gap, 0.5 mm; number of slices, 10; matrix, 256 × 128; number of averages, 20; receiver bandwidth, 50 kHz.

CBF in mice was measured on Day 1 before METH administration and on Day 3 after the last injection of METH. Mice were transferred to the MRI suite, placed in a dedicated holder and moved to the isocenter of the magnet prior to the imaging session. Animals were anesthetized and monitored during the entire duration of the study. Initial localizer images were acquired using the following parameters: 2D FLASH (Fast Low Angle SHot), TR/TE 10/3 ms, matrix 256 × 128, FOV 6.4 cm, 1 slice per orientation. After the localizer images were acquired, tissue perfusion was measured using non-invasive arterial spin labeling method (ASL). The sequence: Flow-sensitive Alternating Inversion Recovery Rapid Acquisition with Relaxation Enhancement (FAIR-RARE) was used to implement ASL with parameters: TE/TR, 46/16,000 ms; FOV, 2.5 cm × 2.1 cm; slice thickness, 1 mm; number of slices, 1; matrix = 128 × 128. Perfusion map was calculated using ASL_Perfusion_Processing macro in ParaVision 5.1 (Bruker Biospin MRI GmbH, Germany). The principle is as follows: Inversion recovery data from the imaging slice are acquired after selective inversion of