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Research Report

Methamphetamine causes sustained depression in cerebral blood flow

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ABSTRACT

The use prevalence of the highly addictive psychostimulant methamphetamine (MA) has been steadily increasing over the past decade. MA abuse has been associated with both transient and permanent alterations in cerebral blood flow (CBF), hemorrhage, cerebrovascular accidents and death. To understand MA-induced changes in CBF, we exposed C56BL/6 mice to an acute bolus of MA (5 mg/kg MA, delivered IP). This elicited a biphasic CBF response, characterized by an initial transient increase (~5 minutes) followed by a prolonged decrease (~30 minutes) of approximately 25% relative to baseline CBF—as measured by laser Doppler flowmetry over the somatosensory cortex. To assess if this was due to catecholamine derived vasoconstriction, phentolamine, an α-adrenergic antagonist was administered prior to MA treatment. This reduced the initial increase in CBF but failed to prevent the subsequent, sustained decrease in CBF. Consistent with prior reports, MA caused a transient increase in mean arterial blood pressure, body temperature and respiratory rate. Elevated respiratory rate resulted in hypocapnia. When respiratory rate was controlled by artificially ventilating mice, blood $PaCO_2$ levels after MA exposure remained unchanged from physiologic levels, and the MA-induced decrease in CBF was abolished. *In vivo* two-photon imaging of cerebral blood vessels revealed sustained MA-induced vasoconstriction of pial arterioles, consistent with laser Doppler flowmetry data. These findings show that even a single, acute exposure to MA can result in profound changes in CBF, with potentially deleterious consequences for brain function.

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Abbreviations: CBF, cerebral blood flow; MA, methamphetamine; Pa, partial pressure; i.p., intraperitoneal; RR, respiratory rate; MAP, mean arterial pressure, HR, heart rate; BT, body temperature; bpm, breath per minute; SEM, standard error of the mean; SD, standard deviation

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1. Introduction

Methamphetamine (MA), a member of the amphetamine class of stimulants, has addictive properties attributable to the activation of dopaminergic reward pathways (Volkow et al., 2001), and as a result has a high potential for abuse. The use of MA is a concerning, rapidly growing epidemic within the United States (Lineberry and Bostwick, 2006). Exposure to MA can cause long-term neurotoxicity and neuroinflammation resulting from mitochondrial dysfunction and oxidative stress due to disruption of catecholamine metabolism (Krasnova and Cadet, 2009). MA exposure produces a myriad of additional physiological responses, both centrally and peripherally, many of which can be attributed to MA induced increases in serotonin, dopamine and norepinephrine (Cruickshank and Dyer, 2009; Sulzer et al., 2005), leading to a pathophysiological increase in sympathetic tone, increased blood pressure, extreme hyperthermia and altered mental state (Schep et al., 2010).

The use of amphetamines, such as MA, is associated with cerebrovascular complications such as cerebrovascular accidents (CVA) (Perez et al., 1999; Westover et al., 2007; Yen et al., 1994), hemorrhage (Delaney and Estes, 1980; Westover et al., 2007), hypoxic damage (Kaye et al., 2008) and vasculitis (Salanova and Taubner, 1984). Interestingly, while changes to cerebral blood flow (CBF) in response to acute amphetamine exposure have been reported (Devous et al., 2001; Rose et al., 2006), there is evidence of long-term effects on CBF from MA use even in abstinent users (Chang et al., 2002; Hwang et al., 2006; Iyo et al., 1997), suggesting that the effect of MA on CBF is at least partially irreversible.

Reports on the effect of MA on global or focal CBF are controversial and incomplete as seen by the variation in published data. While some researchers have reported increases in CBF after amphetamine exposure (Devous et al., 2001; McCulloch et al., 1978; Rose et al., 2006), others have shown that CBF remains unchanged (Kimmerly et al., 2003; Moppett et al., 2008), or is decreased (Alhassoon et al., 2001; Devous et al., 2001; Wang et al., 2001; Zimmer et al., 1974). The discrepancies between studies may be attributed to the substantial variation in methodologies used to measure CBF, such as the examination of venous outflow (Zimmer et al., 1974), SPECT (Devous et al., 2001) or laser Doppler flowmetry (Saeki et al., 1990). It is difficult to compare CBF measured in blood vessels of different caliber (such as middle cerebral artery (Moppett et al., 2008) vs. pial arterioles (Saeki et al., 1990)) since vasomodulatory stimuli differentially affect large and small blood vessels (Kontos et al., 1981). In light of this, we performed a careful analysis of the effect of MA exposure on CBF, and explored possible mechanisms for MA-induced changes in CBF in C57BL/6 mice.

The mouse is not commonly used in such experiments due to the level of technical difficulty encountered during intubation and vascular manipulation in animals of this size. However, mice provide a tractable genetic model system for various neurological diseases and enable facile fluorescent visualization of different cell types within the central nervous system.

2. Results

2.1. *Acute exposure to MA caused sustained decrease in CBF*

CBF was recorded by laser Doppler flowmetry over the somatosensory cortex of anesthetized C57BL/6 mice. After a baseline CBF recording for 5 minutes, MA (5 mg/kg) was injected intraperitoneally (i.p.), and CBF was recorded for 60 minutes. MA exposure caused a bi-phasic response: an initial, transient (<5 minutes) increase in CBF was followed by a prolonged (~30 minutes) decrease, reaching a trough decrease of about 25% relative to baseline flow (Fig. 1A). Injection of 0.9% saline alone did not cause significant changes in CBF (Fig. 1A, shown in grey). The difference between baseline and post-MA levels of CBF was statistically significant (Wilcoxon signed rank test, $p<0.001$). CBF returned to approximate baseline levels within 50 minutes after MA injection.

MA exposure also produced a transient increase in mean arterial pressure (MAP), peaking at about 10 minutes following MA exposure (Fig. 1B). Thus, the peak MA-induced increase in MAP was slightly delayed and prolonged, when compared with the peak initial increase in CBF (which occurred within the first 5 minutes of exposure to MA). MA exposure also produced a sustained elevation in heart rate (HR) that continued even after CBF had returned to baseline (Fig. 1C). The increase in respiratory rate (RR) (Fig. 1D) and body temperature (BT) was consistent with MA-induced stimulation of metabolic rate. The BT increase of approximately 1.5 °C over the course of our experiments (Fig. 1E) is in agreement with well-characterized MA-induced hyperthermia (Cruickshank and Dyer, 2009; Kiyatkin et al., 2007; Rusyniak and Sprague, 2005). Hyperthermia was not seen with administration of saline alone (Fig. 1E, shown in grey). Overall, the MA-induced physiological responses in our experimental mice closely replicated characteristic MA effects in humans, including elevations in HR and MAP (Cruickshank and Dyer, 2009; Gentry et al., 2006; Mendelson et al., 2006) and an increase in RR (Jacobs and Fornal, 1997; Mediavilla et al., 1979).

2.2. *Blocking of α-adrenergic receptors does not prevent the MA-mediated decrease in CBF*

MA is known to reverse the function of the norepinephrine transporter, leading to an increase in norepinephrine levels within the neuronal synapse (Cruickshank and Dyer, 2009). Since the stimulation of sympathetic nerves can cause cerebral vasoconstriction mediated by α_1-adrenergic receptors (Saeki et al., 1990), we tested whether stimulation of α-adrenergic receptors might contribute to the MA-mediated decrease in CBF.

To do this, we treated mice with phentolamine (10 mg/kg, i.p., 30 minutes before MA injection), a nonspecific α-adrenergic antagonist. In mice pretreated with phentolamine, exposure to MA resulted in an attenuated initial increase in CBF (Fig. 2A) that was associated with a reduced rise in MAP (Fig. 2B), compared to mice exposed to MA alone (Fig. 1B). The MA-induced RR increase was not prevented by phentolamine (Fig. 2D), and neither was the MA-induced hypocapnia (Fig. 2E).