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Methamphetamine potentiates behavioral and electrochemical responses after mild traumatic brain injury in mice

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Abstract

We previously demonstrated that high doses of methamphetamine (MA) exacerbate damage induced by severe brain trauma. The purpose of the present study was to examine if MA, at low dosage, affected abnormalities in locomotor activity and dopamine turnover in a mouse model of mild traumatic brain injury (mTBI). Adult male CD1 mice were treated with MA (5 mg/kg i.p.) or vehicle 30-min prior to mTBI, conducted by dropping a 30 g metal weight onto the temporal skull, anterior the right ear. At 15 min after mTBI, animals were put into locomotor activity chambers for up to 72 hours. During the first 3 hours, mTBI alone, compared with vehicle control, did not alter total distance travelled. Treatment with MA significantly increased locomotor activity in the control animals during the first three hours; mTBI reduced MA-induced hyperactivity. In contrast, at 2 and 3 days after injury, mTBI or MA alone reduced locomotor activity. Co-treatment with MA and mTBI further reduced this activity, suggesting a differential and temporal behavioral interaction between MA and mTBI during acute and subacute phases after injury. Dopamine and DOPAC levels in striatal tissue were analyzed using HPLC-ECD. At one hour after mTBI or injection, DA was not altered but DOPAC level and DOPAC/DA turnover ratios were significantly reduced. Co-treatment with MA further reduced the DOPAC/DA ratio. At 36 hours after injury, mTBI increased tissue DA levels, but reduced DOPAC levels and DOPAC/DA ratios. Co-treatment with MA further reduced DOPAC/DA ratios in striatum. In conclusion, our data suggest that low dosage of MA worsens the suppression of locomotor responses and striatal dopamine turnover after mTBI.

Keywords

head trauma; mTBI; dopamine; methamphetamine; behavior

1. Introduction

Traumatic brain injury (TBI) has become an increasingly common cause of brain damage. According to a recent report from the Centers For Disease Control and Prevention, at least 1.4 million people sustain a TBI each year in U.S.A. Recently, there is high incidence of

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TBI amongst soldiers injured in the military operations (Hoge et al., 2008). Several studies have indicated that use of drugs is common in neurotrauma patients. In a systemic review of traumatic brain injury and substance misuse, about 36-51% of patients showed use of some substance on emergency admission to hospital (Parry-Jones et al., 2006).

Methamphetamine (MA), a prevalent drug of abuse, has been found to be associated with brain injury. The use and prevalence of MA have been reported in US military personnel (Klette et al., 2006; Lacy et al., 2008). One study has indicated that 27% of trauma patients used MA; furthermore, these cases were associated with longer hospital stays and hospital charges (Tominaga et al., 2004). Patients with a chronic or acute MA abuse history can develop cerebral hemorrhages in striatum and infarction in the middle cerebral arterial distribution area (Rothrock et al., 1988; Yen et al., 1994). Some patients using the anorexiant phentermine, an analog of amphetamine, developed ischemic stroke (Kokkinos and Levine, 1993). In animal studies, MA was found to activate programmed cell death and facilitate ischemic brain damage (Shen et al., 2008; Wang et al., 2001). Pretreatment with MA potentiates ischemia-mediated P53 expression and cerebral infarction in brain (Wang et al., 2001). High doses of MA induce functional and structural changes in the brain similar to those in TBI (Gold et al., 2009). Taken together, these data suggest that high doses of MA (i.e. the 20-40 mg/kg) potentiate neurodegeneration after severe brain injury (i.e. occlusion of middle cerebral artery). The interaction of low dose of MA and minor insults to the brain has not been reported.

It has been demonstrated that mild traumatic brain injury (mTBI), induced by delivering a 30 g sheer force to the temporal region, did not cause physical signs of damage to skull, scalp and brain in experimental mice (Pan et al., 2003; Zohar et al., 2003). These animals developed a deficiency in cognitive learning ability post-injury (Baratz et al., 2010; Zohar et al., 2003). Similarly, MA at a dose of 5 mg/kg alone does not produce prominent neurotoxicity and has been used to examine locomotor function in hemiparkinsonian rats in numerous studies. The purpose of the present study was to examine the changes in locomotor function as well as the production of dopamine (DA) and its metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) after mTBI and low dose MA treatment in mice. The dose of MA and traumatic injury model used in these studies were closer to human pathophysiological conditions and were thus used to model the interactions of MA and brain injury. Our data suggest that low dose MA exacerbates the suppression of locomotor responses and striatal dopamine turnover after mTBI.

2. Results

2.1 Behavior

Adult male CD1 mice were used for behavior study. Animals were equally divided into 4 groups. (A) MA: These animals received MA injection (5 mg/kg, i.p.). (B) mTBI: Mice received saline injection (1ml/1kg, i.p.) and mTBI at 30 min later. See Methods). (C) mTBI +MA: Mice received MA injection (5 mg/kg, i.p.) and followed by mTBI 30 min later. (D) vehicle control: These animals received only saline injection. All animals were kept in the activity chambers for 3 hours to 3 days with food and water in a room with 12 hour - 12 hour light dark cycle.

Four parameters were used to analyze the changes in locomotor activity. These included total distance travelled, horizontal activity, movement time, and movement number. Behavior was first analyzed every 0.5 hour for 3 hours after injury in 24 mice (Fig 1). There is a significant interaction between various treatments (i.e. MA and mTBI) and time after injury in total distance travelled ($p=0.007$) and movement time ($p=0.001$, Two way ANOVA). During this acute phase, mTBI alone, compared to vehicle control, did not

significantly alter horizontal activity ($p=0.665$), total distance travelled ($p=0.850$), movement time ($p=0.587$), and movement number ($p=0.057$, two way ANOVA+ post-hoc Newman-Keuls test). MA treatment significantly increased all locomotor activity in non-mTBI (i.e. MA vs. veh, $p<0.005$) and mTBI animals (i.e. MA+mTBI vs. mTBI, Table 1, $p<0.005$), especially in the first hour (Fig 1). mTBI significantly reduced MA-mediated hyperactivity (MA vs. MA+mTBI, $p<0.001$, Table 1).

Another set of animals ($n=32$) was used to analyze behavioral response every 4 hours in the sub-acute phase (i.e. 2 and 3 days after injury). Animals demonstrated diurnal motor pattern, i.e. increased activity in dark phase (28-40, 52-64 hours) with reduced activity in light phase (24-28, 40-52, and 64-72 hours). Locomotor activity returned to the same basal level at 24, 48 and 72 hours after injury in the light cycle (Fig 2). The behavior data were analyzed using a two-way ANOVA and post-hoc Newman-Keuls analysis. Either mTBI or MA treatment alone significantly reduced locomotor behavior in all parameters (mTBI vs. veh, $p<0.01$; MA vs. veh, $p<0.01$, Fig 2). No difference was found between the animals treated with mTBI or MA alone ($p>0.3$). Co-treatment with MA and mTBI further reduced locomotor activity, as compared to MA (MA+mTBI vs. MA, Fig 2, Table 1) or mTBI (MA+mTBI vs. mTBI, Fig 2, Table 1) only. These data suggest that MA and mTBI on days 2 and 3 after injury have a synergistic or additive effect on behavior.

2.2 Biochemistry

A total of 56 mice were used to examine DA and DOPAC level in striatum. Animals were sacrificed at 1 ($n=28$) and 36 hours ($n=28$, in dark cycle) after injury. DA, DOPAC, and DOPAC/DA ratio were analyzed using a one way ANOVA and post-hoc Newman-Keuls test.

At one hour after injection or injury, mTBI alone, compared to vehicle treatment, did not alter DA levels (Fig 3A), but significantly reduced DOPAC levels (Fig 3C, $F_{3,24}=17.217$, $p=0.013$) and DOPAC/DA turnover ratios (Fig 3E, $F_{3,24}=38.888$, $p=0.003$). Similarly, MA significantly reduced DOPAC levels (Fig 3C, MA vs. veh, $p<0.001$) and DOPAC/DA ratios (Fig 3E, $p<0.001$). There is a marginal reduction in tissue DA levels after MA treatment, which can only be found by a Student's *t* test (Fig 3A, MA vs. veh, $p=0.030$). There was a further reduction of DOPAC levels in animals treated with MA+mTBI, compared to mTBI only (Fig 3C, $p=0.006$). Co-treatment with mTBI and MA also further suppressed DOPAC/DA ratios (Fig 3E, mTBI+MA vs. mTBI, $p<0.001$; mTBI+MA vs. MA, $p=0.035$) in striatum.

At 36 hours after injection or injury, treatment with mTBI or MA significantly increased tissue DA levels (Fig 3B, $F_{3,24}=10.206$, $p<0.001$). Striatal DA levels were further increased in animals receiving mTBI and MA (Fig 3B, mTBI+MA vs. mTBI, $p=0.039$). mTBI and/or MA also significantly reduced DOPAC levels (Fig 3D, $F_{3,24}=8.027$, $p<0.05$) and DOPAC/DA turnover (Fig 3F, $H=20.882$, $p<0.05$). Co-treatment with mTBI and MA further reduced DOPAC/DA ratios in striatum (Fig 3F, mTBI+MA vs. mTBI, $p<0.001$; mTBI+MA vs. MA, $p=0.035$).

3. Discussion

In this study, we examined the behavioral and biochemical changes elicited by the interaction of MA and mTBI in mice. We found that MA and mTBI have dual effects on locomotor activity in the acute and subacute phases after drug administration. In the acute phase, MA alone increased total distance travelled in the first 3 hours after injection. mTBI did not alter this locomotor activity but suppressed MA-induced hyperactivity. In the subacute phase, MA or mTBI alone reduced locomotor activity. Co-treatment with MA and

mTBI further decrease total distance travelled. These data suggest that there is a differential and time-dependent interaction on locomotor activity after mTBI and MA treatment.

Since MA is an indirect dopaminergic agonist, which releases DA. MA acutely induces behavioral hyperactivity through the release of DA in the striatum. The release of DA from cells is associated with a decrease in tissue DA levels in striatum at one hour after MA administration as seen in our study. We also found that either MA or mTBI reduced DOPAC as well as DOPAC/DA turnover at one hour after injection or injury. As reported previously, the reduction of DOPAC and DOPAC/DA by MA is mediated through the inhibition of MAO or uptake of DA (Qi et al., 2008). There is a further reduction in DOPAC/DA by mTBI in the presence of MA, suggesting mTBI can have synergistic or additive actions with MA on DOPAC/DA turnover acutely.

We found that locomotor activity returned to the same basal level at 24, 48 and 72 hours after injury in the light cycle (Fig 2). Brain tissues were thus collected for DA and DOPAC measurements at 36 hours after injury in the dark period. In contrast to the acute phase, both mTBI and MA treatment increased DA levels in striatal tissue at 36 hours after injury. Previous studies have demonstrated that tyrosine hydroxylase (TH) immunoreactivity was increased in striatum at 28 days after TBI (Yan et al., 2007). No significant increase was found at one day or 1 week after TBI (Yan et al., 2007), suggesting that the increase in tissue DA levels at 36 hours after mTBI seen in present study may not be due to increases in TH protein. However, TH activity can be altered by phosphorylation during insults or nerve stimulation (Masserano et al., 1981; Weiner et al., 1978). Whether mTBI induces phosphorylation of TH requires further investigation. It has been reported that dopamine clearance and dopamine transporter expression were suppressed 2 weeks after TBI (Wagner et al., 2005). Our data also showed that DOPAC levels and DOPAC/DA ratios were suppressed by mTBI, suggesting that the turnover or metabolism of DA was suppressed by mTBI as early as 36 hours after injury. It is also likely that the increase in tissue DA level may be secondary to a reduction of DA turnover after mTBI and MA treatment. We found that MA + mTBI reduced locomotor activity in subacute period. Similarly, DOPAC/DA turnover was further suppressed by MA in mTBI mice at 36 hours. These data suggest that MA exacerbates mTBI-mediated bradykinesia, which may be attributed to the reduction in DOPAC/DA turnover.

In this study, we applied MA, 30 min before mTBI, to simulate the clinical situation of MA use before mTBI. We found that MA exacerbates mTBI-mediated bradykinesia and further reduced DOPAC/DA turnover in striatum at 2 days after mTBI. Several studies have shown that changes in nigrostriatal dopamine levels or its turnover occurs at the presymptomatic phase after nigrostriatal lesioning, such as in Parkinson's disease models. It is thus likely that a change in DOPAC/DA turnover is not a result, but a cause, of bradykinesia after MA and mTBI injury.

We previously reported that high doses of MA exacerbate damage induced by severe brain trauma (Wang et al., 2001). Pretreatment with MA (40 mg/kg) potentiates the expression of p53 mRNA and volume of infarction in mice after stroke. A recent review from Gold et al also demonstrated similar proteomic changes with TBI and with acute high dose MA treatment (Gold et al., 2009). These data suggest a potential interaction between the use of high dose MA and brain trauma, possibly through an apoptotic mechanism. In this study, we demonstrated that MA, at much lower dosage (5mg/kg), potentiates an mTBI-mediated biochemical and behavioral responses at 36 hours after injection. Our data suggest that low dose MA has a prolonged action causing behavioral impairment and suppressing DA turnover at 36 hours after minor brain injury. Several clinical and epidemiological reports have indicated the use of this drug is common in neural trauma patients. For example, use of

MA or its analogs was associated with driving impairment and fatality in patients (Gustavsen et al., 2006; Logan, 1996; Verschraagen et al., 2007). Data from CDC's report "traumatic brain injury in the United States" indicated that "vehicle traffic" is the second leading cause of TBI. A synergistic/additive action between MA and mTBI may thus be involved in these patients. In conclusion, our data suggest that low doses of MA can potentiate mTBI-mediated suppression of dopamine turnover and behavioral immobilization. Exposure to low doses of MA increases functional deficits after mild traumatic brain injury.

4. Experimental Procedures

4.1 Animals and mTBI

Adult CD1 mice, purchased from Charles River Laboratories Inc., were housed in a 12 hour dark (6pm to 6 am) and 12 hour light (6 am to 6 pm) cycle. Animals were treated with saline (1ml/kg) or (+) methamphetamine (5mg/kg), and were anesthetized with isoflurane at 30 minutes following drug administration for mTBI. mTBI was conducted by dropping a 30 g metal projectile onto the temporal skull, anterior the right ear as previously described (Pan et al, 2003; Zohar et al, 2003). Anesthetized mice were laid on their side. A metal tube (13 mm in inner diameter) was placed vertically over the head and a metal projectile was dropped from 80 cm height down the tube to strike the temporal region of skull anterior to the right ear. The rod-shaped projectile was made of metal with a slightly rounded end in order to enable a smooth contact with the skull without any external damage at the site of the weight drop. A sponge immobilization pad (L:4-5 in; W: 2.7 in ; H: 1.8 in) was employed; this allows head movements during the injury. Animals in control groups were anesthetized for the same duration.

4.2 Locomotor behavioral measurement

Animals recovered from gas anesthesia in 5 min after mTBI. At 15 min after injury, animals were individually placed in the locomotor activity chambers (Accuscan, Columbus, OH) for up to 3 days (12-hr light and 12-dark/day). Food and water were constantly provided in the chambers, which contained 16 horizontal and 8 vertical infrared sensors spaced 2.5 cm apart. Each animal was placed in a 42 × 42 × 31 cm plexiglass open box. Motor activities were measured by the number and order of beams broken by the animals.

4.3 Striatal Dopamine and DOPAC measurement

Brain samples were collected at 1 and 36 hours after injury from a subgroup of animals. Striatal tissues were weighed and stored at -80°C until extraction. The tissues obtained from each animal were homogenized in 0.1 M perchloric acid and centrifuged at 13,000 g for 15 min. DA and DOPAC were measured by HPLC with electrochemical detection. The analytical column was a Symmetry C18 3.5 µm (4.6×150.0 mm) from Waters (Milford, MA). The mobile phase consisted of 0.01 M sodium dihydrogenphosphate, 0.01 M citric acid, 2 mM sodium EDTA, 1 mM sodium octylsulfate, 10% methanol, pH 3.5 and was used at flow rate of 0.9 ml/min. The system consisted of an ESA automated injection system, an ESA 582 pump, and a Coulochem III detector (ESA, Chelmsford, MA). An EZChrom Elite™ chromatography data analysis system (ESA Biosciences, Inc.) was used for data collection and analysis. Contents of DA and DOPAC were calculated as nmole/g of tissue weight.

4.4 Statistical analysis

All data were expressed as means $\pm$ SEM. Behavioral and biochemical data were analyzed using two or one way ANOVA and post-hoc Newman-Keuls test. All analyses were calculated by SigmaStat software ver 3.0. Statistical significance was preset at $p < 0.05$.

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Abbreviations used in this paper

mTBI	Mild traumatic brain injury
MA	Methamphetamine
DA	Dopamine
DOPAC	3,4-dihydroxyphenylacetic acid

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Fig. 1. Locomotor activity in the acute phase (initial 3 hours) after treatment with MA and/or mTBI. Behavioral data were analyzed every 30min. Treatment with MA (5mg/kg) enhanced (A) total distance travelled, (B) horizontal activity, (C) movement time, and (D) movement number. mTBI did not alter locomotor activity, but significantly reduced MA -mediated hyperactivity. ($p < 0.05$, two way ANOVA+Newman-Keuls test).

Fig. 2.

Locomotor activity in the sub-acute phase (2 and 3 days) after treatment with MA and/or mTBI. Behavioral data were analyzed every 4 hours. Animals demonstrated increased activity in dark phase with reduced activity in light phase. Locomotor activity returned to the same basal level at 24, 48 and 72 hours after injury in the light cycle. mTBI or MA alone reduced activity. Co-treatment with MA and mTBI further significantly attenuated locomotor activity, as compared to treatment with MA or mTBI only ($p < 0.05$, two way ANOVA+Newman-Keuls test).

Fig. 3.

Striatal DA, DOPAC and DOPAC/DA ratios in mice treated with MA and/or mTBI. At one hour after injection or injury (A, C, E), mTBI, compared to vehicle treatment, significantly reduced DOPAC levels and DOPAC/DA turnover ratios. MA alone significantly reduced tissue DA and DOPAC levels as well as DOPAC/DA ratios. There was a further reduction of DOPAC levels and DOPAC/DA ratios in animals treated with MA +mTBI, compared to mTBI only. At 36 hours after injection or injury (B, D,F), treatment with mTBI or MA significantly increased tissue DA level ($p<0.001$). Striatal DA levels were further enhanced in mice treated with mTBI and MA. mTBI and/or MA significantly reduced DOPAC levels and DOPAC/DA turnover. Co-treatment with mTBI and MA further suppressed DOPAC/

DA ratios in striatum. (# $p=0.030$, Student's t test; * $p<0.05$, one way ANOVA+Newman-Keuls test)

Table 1

Difference in locomotor activity between animals treated with MA+mTBI and vehicle, MA, or veh+mTBI.

	0-3 hr		24-72hr			
	p value		F value	p value		F value
	MA+mTBI vs.		MA+mTBI		MA vs.	
	veh	MA	veh+mTBI	veh	MA	veh+mTBI
Horizontal activity	0.01	<0.001	0.001	<0.001	0.005	0.004
Total distance travelled	<0.001	<0.001	<0.001	<0.001	0.049	0.043
Movement number	0.151	<0.001	0.002	<0.001	0.024	0.083
Movement time	0.002	<0.001	<0.001	<0.001	0.03	0.056
						12.806

P and F values were calculated using a two-way ANOVA and post-hoc Newman-Keuls test.

Horizontal activity = total number of beam interruptions that occurred in the horizontal sensors

Total distance travelled = the total distance (cm) travelled.

Movement number = the number of separate horizontal movements executed.

Movement time = the amount of time (second) in ambulation.