Amphetamine causes dopamine depletion and cell death in the mouse olfactory bulb

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Abstract

Amphetamine is a neurotoxic psychostimulant that causes dopamine depletion and neuronal death in the rodent striatum. In the present study, we sought to determine if toxic doses of the drug can also induce pathological changes in the mouse olfactory bulb. We found that injections of amphetamine (10 mg/kg × 4, given 2 h apart) caused significant decreases in dopamine levels in that structure. This dose of the drug also induced substantial increases in the number of terminal deoxynucleotidyl transferase-mediated deoxyribonucleotide triphosphate (dNTP) nick end labeling (TUNEL)-positive cells in the olfactory bulb indicative of elevated DNA fragmentation. These results show that the toxic effects of amphetamine involve the olfactory bulb in addition to the striatum. These observations need to be taken into consideration when discussing the clinical course of amphetamine addiction.

Keywords

neurotoxicity; striatum; TUNEL staining; DNA fragmentation

1. Introduction

Amphetamine is a psychostimulant which is abused by a large sector of the world population (Boniatti et al., 2007; Holmgren et al., 2007; Teter et al., 2006). Its abuse is accompanied by many medical and neuropsychiatric complications, which include impairments of attention, memory and decision-making (McKetin and Mattick, 1997; Ornstein et al., 2000; Rogers et al., 1999). The neurotoxic effects include long-term dopamine depletion (Wagner et al., 1980) and loss of dopamine transporters (Krasnova et al., 2001). In addition, injections of the drug also cause cell death in the cortex of amphetamine-treated animals as was shown by Fluoro-Jade staining and electron microscopy (Jakab and Bowyer, 2002; Ryan et al., 1990). More recently, Krasnova et al. (2005) reported that the use of amphetamine is associated with neuronal death in the mouse striatum.

It was, however, not clear to what extent amphetamine might also cause cell death in other brain regions such as the olfactory bulb, which is rich in dopamine neurons (Davila et al., 2003; Philpot et al., 1998). Therefore, we sought to determine if injections of amphetamine doses, that are known to cause dopamine depletion and cell death in the striatum, can also induce similar neuropathological changes in the mouse olfactory bulb.

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2. Materials and methods

2.1. Animals and amphetamine treatment

Male CD-1 mice 10–12 weeks old, weighing 30–35 g and obtained from Charles River (Raleigh, NC) were used in the study. All animal procedures were performed according to the NIH Guide for the Care and Use of Laboratory Animals and were approved by the local Animal Care Committee. The mice were injected with d-(+) amphetamine sulfate (10.0 mg/kg $\times$ 4 times, every 2 hours) or saline and euthanized at different time intervals after treatment.

2.2. High-performance liquid chromatography analysis

To examine amphetamine-induced depletion of monoaminergic terminals in the mouse brain, two groups of animals were sacrificed 1 week and 2 weeks after drug treatment. Olfactory bulb and striatal tissue samples (N = 6–10 per group) were dissected from each brain, weighed and extracted with 0.1 M perchloric acid, then centrifuged at 20,000g for 10 min. Concentrations of dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), serotonin (5-HT), and 5-hydroxyindoleacetic acid (5-HIAA) in the tissue extracts of amphetamine- and saline-treated mice were measured by high-performance liquid chromatography with electrochemical detection as described earlier (Krasnova et al., 2000) and expressed as ng/mg of tissue weight.

2.3. Terminal deoxynucleotidyl transferase-mediated deoxyribonucleotide triphosphate (dNTP) nick end labeling (TUNEL) histochemistry

To test if amphetamine causes cell death in the mouse brain, we performed TUNEL histochemistry that detects DNA fragmentation using the protocol previously described by us (Krasnova et al., 2005). At 3 and 4 days after amphetamine or saline injections, animals were euthanised, their brains were removed and frozen in isopentane on dry ice. The brains were then cut into 30gm coronal sections on the cryostat, and sections containing striatal and olfactory bulb areas were used for TUNEL staining. For statistical analyses, TUNEL-positive cells were counted in coronal sections using a Zeiss (Carl Zeiss MicroImaging, Inc., Thornwood, NY) microscope. Five sections were counted per brain region from each mouse, and 5–8 mice were used in each group.

2.4. Statistical Analysis

All data are presented as means ± SEM. Statistical analysis was performed using analysis of variance followed by Fisher's protected least significant difference (StatView 4.02, SAS Institute, Cary, NC). Differences were considered significant at $p \leq 0.05$.

3. Results

In order to test the effects of the drug on the olfactory bulb, we used amphetamine doses that have been shown to cause decreases in striatal dopamine levels (Krasnova et al., 2001). Injections of these doses of the drug do indeed cause substantial reductions in dopamine concentrations in both the olfactory bulb (Fig. 1A) and striatum (Fig 1B). The changes in dopamine levels corresponded to 80% and 20% decreases in the striatum and olfactory bulb, respectively, and lasted for the two weeks of observations. Amphetamine also causes marked decreases (about −60%) in DOPAC levels in the striatum (Fig 1B) but not in the olfactory bulb (Fig. 1A). Similarly, the drug also induced reductions (− 45%) in HVA levels in the striatum without changing those in the olfactory bulb (data not shown). DOPAC/dopamine ratios were increased in both brain regions at 1 week after amphetamine injections (Fig. 1A, B), but went back to normal in the olfactory bulb at 2 weeks post-drug (Fig. 1A). Amphetamine treatment

had no effect on 5-HT and 5-HIAA levels in the olfactory bulb and striatum at any of the time-points studied (data not shown).

Next, we performed TUNEL histochemistry to examine if amphetamine could cause cell death in the olfactory bulb. Previous study that investigated time-course of amphetamine-induced cell death in the striatum had shown that it peaked at 4 days after drug treatment (Krasnova et al., 2005). Therefore, we chose 3 and 4 day intervals to perform TUNEL staining in the olfactory bulb and striatum. Fig. 2 shows the effects of the drug on DNA fragmentation in the two brain regions. In agreement with previous findings (Deng et al., 2007), we found some TUNEL-positive cells in the olfactory bulb (Fig. 2A), but very few of those in the striata of control mice (Fig. 2B). Amphetamine caused significant increases in the number of TUNEL-positive cells in both the olfactory bulb (Fig. 2A) and the striatum (Fig. 2B). The number of drug-induced TUNEL-positive cells peaked at 3 days in the olfactory bulb (Fig. 2A) but at 4 days in the striatum (Fig. 2B).

4. Discussion

Our main observations are that amphetamine can cause depletion of dopamine levels and significant increases in the number of TUNEL-positive cells in the mouse olfactory bulb. These findings are consistent with results of previous studies showing that the more potent psychostimulant, methamphetamine, can also cause degeneration of dopamine terminals and neuronal death in various regions of the rodent brain including the olfactory bulb (Deng et al., 2007). Although amphetamine and methamphetamine are both abused drugs that share similar chemical structure and cause dopamine release in the striatum, several studies have demonstrated differences in their neurochemical and behavioral effects (Peat et al., 1985; Ricaurte et al., 1980; Shoblock et al., 2003a,b; Wagner et al., 1980). Specifically, amphetamine, but not methamphetamine, can cause increase in glutamate levels in the nucleus accumbens; in contrast, methamphetamine causes elevation in glutamate concentrations in the prefrontal cortex whereas amphetamine has no effects (Shoblock et al., 2003b). In addition, amphetamine causes greater locomotor activity (Shoblock et al., 2003b) and induces more marked impairments in rat working memory (Shoblock et al., 2003a). Studies assessing the neurotoxic effects of the two drugs have shown that methamphetamine treatment results in depletion of dopamine and serotonin terminals in the brains of C57BL/6J mice (Ladenheim et al., 2000) and rats (Peat et al., 1985; Ricaurte et al., 1980), whereas amphetamine causes decreases in dopamine concentrations without affecting serotonin levels in these animals (Krasnova et al., 2001; Peat et al., 1985; Wagner et al., 1980). Thus, our data expand the literature comparing the neurotoxicity of the two drugs and provide documentation that they have similar toxic effects on the olfactory bulb.

Structurally, the olfactory bulb contains five layers. These include the subependymal, the combined mitral and granule cell, the external plexiform and the glomerular layers (Lledo et al., 2006). Because dopaminergic neurons are abundantly expressed in the glomerular layer of the olfactory bulb (Davila et al., 2003; Halasz et al., 1981), the present findings that amphetamine can cause dopamine depletion in this brain area suggests that the drug does indeed have effects beyond the nigrostriatal dopaminergic projections in the forebrain (Krasnova et al., 2001). These observations are consistent with the idea that the toxic effects of amphetamine are mediated by dopamine-dependent generation of oxygen-based radicals subsequent to psychostimulant-induced redistribution of the neurotransmitter from synaptic vesicles to the cytosol (Cadet et al., 2007; Fleckenstein et al., 2007). Amphetamine-induced increases in TUNEL-positive cells in the olfactory bulb are probably due to similar mechanisms, with secondary DNA breakdown being caused by dopamine-generated quinones, hydroxyl (Huang et al., 1997) or superoxide (Krasnova et al., 2001) radicals.

Evidence has accumulated to show the important role for dopamine in olfaction (Hsia et al., 1999). Specifically, dopamine inhibits transmission between olfactory epithelium and the glomeruli of the olfactory bulb, mediating the entry of olfactory information into the brain (Hsia et al., 1999). Therefore, dopamine depletion in the olfactory bulb after amphetamine treatment may induce impairments in olfaction because low dopamine levels would cause disinhibition of olfactory glomeruli leading to disorganized olfactory processing. This idea is in agreement with clinical studies showing that patients who suffer from Parkinson's disease experience hyposmia that precedes motor deficits (Hawkes, 2003). While Parkinson's disease patients suffer from degeneration of dopamine neurons in the substantia nigra, there is a significant increase in the number of dopaminergic cells in the olfactory bulb (Huisman et al., 2004). Because dopamine can inhibit olfactory transmission (Hsia et al., 1999), the increased dopamine levels in the olfactory bulb were hypothesized to underlie hyposmia in Parkinsonian patients (Huisman et al., 2004). Since amphetamine causes the opposite effects in the olfactory bulb decreasing dopamine levels, it suggests that drug-treated rodents and human abusers might suffer from hyperosmia. Clinical studies of olfaction in amphetamine abusers are needed to test this idea.

Because olfactory bulb is a part of the limbic system, it is also involved in motivation, emotions and memory (Rolls, 2005). Indeed, olfactory bulbectomy in rodents causes reward deficits similar to depressive symptoms in humans and is used as an animal model of depression (Song and Leonard, 2005). It is interesting that both depression and amphetamine withdrawal in humans are associated with diminished interest in reward stimuli and accumulated data suggest potential common neurobiological substrates underlying depressive states observed in these psychiatric conditions (Paterson and Markou, 2007). While olfactory dysfunction has been reported in patients with depression (Pause et al., 2001; Satoh et al., 1996), the association of this phenomena with depressive-like symptoms related to amphetamine withdrawal remains to be evaluated.

Acknowledgements

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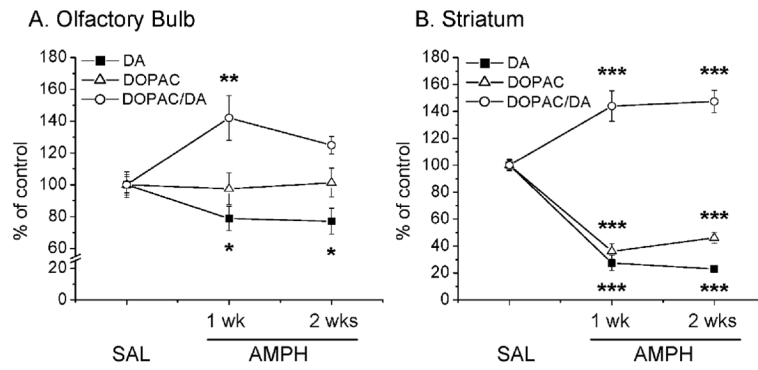
Figure 1.
Effects of amphetamine (AMPH) administration on the dopamine (DA), DOPAC levels and DOPAC/DA ratios in the olfactory bulb (A) and striatum (B). N = 6–10 animals per group. Key to statistics: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$ in comparison to saline-treated control.
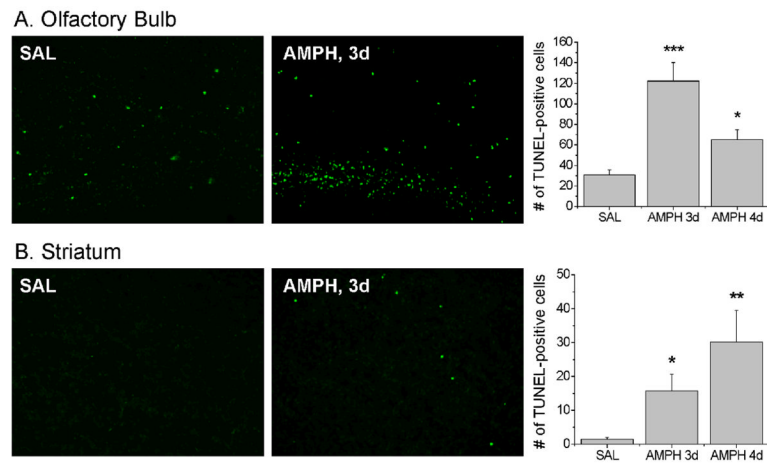
Figure 2.
Effects of AMPH treatment on DNA fragmentation in the ofactory bulb (A) and striatum (B). TUNEL staining of coronal sections from saline- and AMPH-treated mice is shown. N = 5–8 animals per group. Key to statistics: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$ in comparison to saline-treated control.