Methamphetamine self-administration attenuates hippocampal serotonergic deficits: role of brain-derived neurotrophic factor

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Abstract
Preclinical studies suggest that prior treatment with escalating doses of methamphetamine (METH) attenuates the persistent deficits in hippocampal serotonin (5-hydroxytryptamine; 5HT) transporter (SERT) function resulting from a subsequent ‘binge’ METH exposure. Previous work also demonstrates that brain-derived neurotrophic factor (BDNF) exposure increases SERT function. The current study investigated changes in hippocampal BDNF protein and SERT function in rats exposed to saline or METH self-administration prior to a binge exposure to METH or saline. Results revealed that METH self-administration increased hippocampal mature BDNF (mBDNF) immunoreactivity compared to saline-treated rats as assessed 24 h after the start of the last session. Further, mBDNF immunoreactivity was increased and SERT function was not altered in rats that self-administered METH prior to the binge METH exposure as assessed 24 h after the binge exposure. These results suggest that prior exposure to contingent METH increases hippocampal mBDNF, and this may contribute to attenuated deficits in SERT function.

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Introduction
Methamphetamine (METH) abuse is a serious worldwide health problem. METH abusers often display structural and neurochemical changes within the brain and higher rates of psychiatric disorders and cognitive deficits (for review, see Chang et al., 2007; Panenka et al., 2013). Specifically, positron emission tomography has indicated decreased serotonin (5-hydroxytryptamine; 5HT) transporter (SERT) densities in several brain regions in abstinent METH users which were associated with an increased magnitude of aggression (Seke et al., 2006). However, post-mortem studies found no significant decreases in SERT immunoreactivity in the hippocampus of METH users (Kish et al., 2009). These studies emphasize the need for study of the neurochemical changes in the hippocampus following METH exposure.

Recent studies have suggested that prolonged escalating-dose pretreatments by either noncontingent or contingent administration more closely models some aspects of human METH abuse (Krasnova et al., 2013; Lačan et al., 2013). Of note these dosing paradigms often lead to attenuated persistent deficits compared to those animals only given METH in a ‘binge-like’ pattern (e.g. 3–6 injections, 7.5–50 mg/kg/injection, 2–8-h intervals; Johnson-Davis et al., 2003; Belcher et al., 2008; Cadet et al., 2009; McFadden et al., 2012a,b). Specifically, escalating doses of METH results in attenuated persistent deficits in 5HT content and SERT binding in the hippocampus following a binge exposure (Johnson-Davis et al., 2003; Belcher et al., 2008). Prior self-administration of METH that leads to escalating METH intake also attenuates the persistent deficits induced by a binge METH exposure in the hippocampus and striatum (McFadden et al., 2012a, b). Despite these findings, the mechanism underlying this protection is not clearly understood.

Brain-derived neurotrophic factor (BDNF) has been shown to regulate SERT function (Bemmansour et al., 2008). Infusions of BDNF increase SERT function in the hippocampus as measured by in vivo chronoamperometry and microdialysis (Bemmansour et al., 2008). Further, BDNF can increase terminal sprouting in serotonergic neurons following a serotonergic lesion (Mamounas et al., 2000). These findings suggest that BDNF can influence the serotonergic system.

BDNF is up-regulated following insults to the central nervous system (Braun et al., 2011). Whereas the mature form of BDNF (mBDNF) promotes the growth, development, differentiation and maintenance of neuronal...
systems, neuronal plasticity, synaptic activity and neurotransmitter-mediated activities, the precursor of BDNF (proBDNF) when bonded to the p75 neurotrophin receptor promotes apoptosis (Koshimizu et al., 2010). Rodent studies have found METH alters BDNF mRNA in various regions of the brain (Cadet et al., 2009, 2011; Braun et al., 2011). Recently, time-dependent changes in BDNF proteins were found in the dorsal striatum following METH self-administration (Krasnova et al., 2013). Similarly, human METH users often have elevated blood serum levels of BDNF (Kim et al., 2005). Of note, BDNF levels decreased with prolonged abstinence in human psychostimulant users (Hilburn et al., 2011). These findings suggest that BDNF may play a role in the neurochemical changes following METH use. Therefore, the purpose of the current study was to investigate the changes in BDNF as they relate to changes in serotonergic function in hippocampus following METH self-administration and a binge exposure to the drug.

Methods

Animals

Adult male Sprague–Dawley rats (275–300 g; Charles River Laboratories, USA) were housed four rats per cage. Following surgery, each rat was individually housed in a transparent plastic cage. Water was available in their home cage ad libitum. During food training, rats were food restricted such that no rat dropped below 90% of their body weight at the time the experiment was initiated. Rats were maintained under the same 14:10 h light/dark cycle in the animal facility and in the operant chambers. Animals were sacrificed by decapitation. All experiments were approved by the University of Utah’s Institutional Animal Care and Use Committee, in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Self-administration and METH challenge

Food training and self-administration occurred in an operant chamber (Coulbourn Instruments, USA) as described in McFadden et al. (2012a, b). Rats underwent 7 d of self-administration (8 h/session; FR1; 0.12 mg/infusion racemic-METH expressed as free-base or saline; generously supplied by the National Institute on Drug Abuse, National Institute of Health, USA) during the light cycle as previously described (McFadden et al., 2012a, b). For each active lever press, an infusion pump delivered 10 μl of METH or saline over a 5-s duration. During this period, both levers were retracted. Following the infusion, the levers remained retracted for an additional 20 s. Pressing the inactive lever resulted in no programmed consequences, although it was recorded. Animals were sacrificed 24 h after the start of the last self-administration session or received a binge of METH or saline. Twenty-four hours after the start of the last self-administration session, rats were challenged with four injections of METH (7.5 mg/kg/injection; 2-h interval) or saline (1 ml/kg/injection) as described previously (McFadden et al., 2012a, b). Animals were sacrificed 24 h after the binge exposure.

Synaptosomal [3H]5HT uptake

[3H]5HT uptake was determined using a rat hippocampal synaptosomal preparation as previously described (McFadden et al., 2012b). In brief, synaptosomes were prepared by homogenizing freshly dissected hippocampal tissue in ice-cold 0.32 m sucrose buffer (pH 7.4), and centrifuged (800 g, 12 min; 4 °C). The supernatants were centrifuged (22,000 g, 15 min; 4 °C) and the resulting pellets were resuspended in ice-cold assay buffer (in mM: 126 NaCl, 4.8 KCl, 1.3 CaCl2, 16 sodium phosphate, 1.4 MgSO4, 11 glucose and 1 ascorbic acid; pH 7.4) and 1 μM pargyline. Samples were incubated for 10 min at 37 °C and the assays initiated by the addition of [3H]5HT (5 nM final concentration). Following incubation for 3 min, samples were placed on ice to stop the reaction. Samples were then filtered through GF/B filters (Whatman, USA) soaked previously in 0.05% polyethylenimine. Filters were rapidly washed three times with 3 ml of ice-cold 0.32 m sucrose buffer using a filtering manifold (Brandel, USA). Nonspecific values were determined in the presence of 10 μM fluoxetine. Radioactivity trapped in filters was counted using a liquid scintillation counter. Protein concentrations were determined using the Bio-Rad Protein Assay (Bio-Rad Laboratories Inc., USA).

Western blotting

Equal quantities of protein (10 μg) of hippocampal synaptosomes were loaded into each well of a 4 to 12% NuPAGE Novex Bis-Tris Midi gradient gel (Invitrogen, USA) and electrophoresed using a XCell4 SureLock Midi-cell (Invitrogen). Membranes were blocked for 45 min with Starting Block Blocking Buffer (Pierce Chemical, USA), and electrophorosed using a XCell4 SureLock Midi-cell (Invitrogen). Membranes were blocked for 45 min with Starting Block Blocking Buffer (Pierce Chemical, USA) and incubated for 1 h at room temperature with an anti-BDNF polyclonal antibody (N-20; 1:1000; Santa Cruz Biotechnology, USA) and incubated for 1 h at room temperature with an anti-BDNF polyclonal antibody (N-20; 1:1000; Santa Cruz Biotechnology, USA). The polyvinylidene difluoride membrane was then washed five times in Tris-buffered saline with Tween (250 mM NaCl, 50 mM Tris, pH 7.4, and 0.05% Tween 20). The membranes were then incubated for 1 h with a horseradish peroxidase-conjugated secondary antibody (BioSource International, USA). After five washes in Tris-buffered saline with Tween, the bands (mBDNF-14 kDa and its precursor proBDNF-32 kDa; Molteni et al., 2009) were visualized using Western Lightning Chemiluminescence Reagents Plus (PerkinElmer Life and Analytical Sciences) and were quantified by densitometry using a FluorChem SP Imaging System (Alpha Innotech, USA). Membranes were then stripped with Restore Western Blot Stripping Buffer (Thermo Scientific, USA), blocked for 45 min with Starting Block Blocking Buffer (Pierce Chemical, USA), and incubated for 1 h at room temperature.
temperature with an anti-β-Actin primary antibody (13E5, Cell Signaling Technology, USA). Membranes were then washed, incubated in secondary antibody, washed, and visualized (β-Actin - 45 kDa) as described above. Protein concentrations were determined using the Bradford Protein Assay.

Statistical analysis

Statistical analysis was conducted in GraphPad Prism (USA). Statistical analyses among groups were conducted using a t-test or analysis of variance (ANOVA) followed by Newman-Keuls posthoc analyses. The data represent means±S.E.M. of 6–14 rats/group.

Results

Results presented in Fig. 1a, b indicate that METH self-administration increased hippocampal mBDNF (t(13)=3.197, p<0.05), but not proBDNF (t(13)=1.343, ns) immunoreactivity or β-Actin immunoreactivity (t(13)=0.78, ns; Saline: 1062±102.20 arbitrary units; METH: 1168±89.12 arbitrary units) as assessed 24 h after the last self-administration session. Previous reports have shown SERT function was unaltered at this time point in METH self-administering rats (t(12)=0.62, ns; Fig. 1c; from McFadden et al., 2012b with permission). This time point corresponded with the first injection of METH during the multiple METH exposure. During self-administration, rats self-administered 2.38±0.17 mg METH on D1 and increased intake to 3.67±0.31 mg METH on D7.

Similarly, when animals were sacrificed 24 h after the last injection of the binge exposure, no decreases in SERT function (Fig. 1e) and an increase in mBDNF (Fig. 1d) occurred in METH/METH animals compared to the saline control animals. Only rats that had previously self-administered saline prior to the binge METH exposure had a significant decrease in SERT function (F(2,17)=5.792, p<0.05) compared to the Saline/Saline group. The lack of significant decreases in SERT function 24 h after the binge exposure in the METH/METH group was associated with a significant increase in mBDNF immunoreactivity (F(2,16)=4.405, p<0.05; Fig. 1d), but no significant change in proBDNF immunoreactivity (F(2,16)=0.116, ns; Fig. 1d) or β-Actin immunoreactivity (F(2,16)=0.58, ns; Saline/Saline: 963.50±95.46 arbitrary units; METH/METH: 906.30±39.60 arbitrary units; Saline/METH: 867.20±41.40 arbitrary units).
units). During self-administration, rats self-administered 2.13±0.22 mg METH on D1 and increased to 3.44±0.26 mg METH by D7.

**Discussion**

Previous reports have shown prior METH exposure can attenuate persistent serotonergic deficits in the hippocampus following a binge exposure to the drug (Johnson-Davis et al., 2003; Belcher et al., 2008; McFadden et al., 2012b). No significant decrease in SERT function was observed 24 h following the binge METH exposure in the animals that were allowed to self-administer METH prior to the binge. The increase in SERT function 24 h after the binge METH exposure in the METH/METH group compared to the Saline/METH may contribute to attenuations of the persistent neurotoxic deficits in hippocampal SERT function or binding and 5HT content afforded by prior escalating dose METH exposures (Johnson-Davis et al., 2003; Belcher et al., 2008; McFadden et al., 2012b).

It is possible that an increase in mBDNF contributes to the elevated SERT function 24 h after the binge exposure. Both intracerebroventricular and local infusions of BDNF increase SERT function in the hippocampus as measured by in vivo chronoamperometry and microdialysis (Benmansour et al., 2008). Of note, these changes were seen within 30–120 min following BDNF infusions and were not associated with increases in SERT binding densities, suggesting that these are acute changes in SERT function (Benmansour et al., 2008). Further evidence for the role of BDNF in regulating the SERT function comes from heterozygous BDNF mice that have lower BDNF protein levels (Daws et al., 2007). Adult 5 and 10 month old (+/−) BDNF have reduced rates of 5HT clearance in the hippocampus compared to wild-type mice but have similar SERT binding (Daws et al., 2007). Similarly, 3–4 month old (+/−) BDNF mice have reduced synaptosomal SERT uptake compared to wild-type mice in the hippocampus (Guiard et al., 2008). These findings suggest that BDNF can affect SERT functioning.

In light of the previous research, the results of the present study suggest that increases in mBDNF protein by METH self-administration can contribute to the regulation of SERT function. Of interest, previous studies have reported no significant difference in SERT function 24 h or 8 d after the start of the last self-administration session (McFadden et al., 2012b), but slight decreases in SERT immunoreactivity following METH self-administration (Reichel et al., 2012). The increase in mBDNF following METH self-administration may have increased SERT function thus resulting in no differences in SERT function (McFadden et al., 2012b) but differences in SERT immunoreactivity (Reichel et al., 2012). Further, the significant increase in mBDNF in the animals that received a binge of METH following METH self-administration may contribute to the lack of significant decreases of SERT function 24 h after the binge exposure to METH. It can be speculated that the observed changes in mBDNF following contingent METH exposure may be a contributing factor underlying METH-induced tolerance to serotonergic deficits.

Previous studies have shown trophic factors can increase terminal sprouting in serotonergic neurons (Mamounas et al., 2000). For example, the administration of BDNF into the hippocampus promoted the regenerative sprouting of serotonergic axons with a pattern of reinnervation reminiscent of normal 5-HT innervation when given 1 wk following a neurotoxic para-chloroamphetamine (PCA) exposure (Mamounas et al., 2000). It was suggested that the sprouting of these terminals was due in part to a retrograde signaling mechanism which takes between 4–7 d to initiate sprouting (Mamounas et al., 2000). It is unknown if the increases in mBDNF found in the METH/METH group 24 h following the binge exposure persists and therefore may help promote sprouting of serotonergic terminals to attenuate persistent deficits. Future studies are required to investigate this.

These findings are consistent with reports by Marshall and colleagues in that wheel running can attenuate persistent serotonergic deficits as a result of binge exposure to METH (O’Dell et al., 2012). Voluntary exercise has been previously shown to increase trophic factors in the hippocampus including BDNF (Griesbach et al., 2008). Wheel running prior to and following the binge exposure to METH may increase trophic factors including mBDNF similar to that of METH self-administration and result in decreased persistent serotonergic deficits including losses in SERT function/binding and 5HT content following a binge exposure to METH, similar to what was observed in the current study.

In summary, prior METH exposure through escalating doses or self-administration decreases persistent serotonergic deficits within the hippocampus (Johnson-Davis et al., 2003; Belcher et al., 2008; McFadden et al., 2012b); however, the mechanism underlying this phenomenon is unknown. The findings of the current study suggest that prior METH self-administration increases mBDNF in the hippocampus, perhaps as a compensatory mechanism, which may in turn influence SERT function and the response of serotonergic systems to a neurotoxic METH administration. Because previous studies suggested that BDNF can increase SERT function, it can be speculated that BDNF may be responsible for the increase in SERT function in the METH/METH group compared to the Saline/METH group. The observed increase in mBDNF in the METH/METH group may have also lead to an increase in serotonergic axonal sprouting at later time points and thus resulted in the attenuated decreases in persistent serotonergic deficits induced by the binge exposure to METH as previously reported (McFadden et al., 2012b). The putative role of key BDNF-associated pathways including tyrosine receptor kinase B, phosphorylated extracellular signal-regulated kinases (ERK)/total ERK ratios, and phospho-AKT/total AKT ratios.
(Koshimizu et al., 2010) in these phenomena remains to be explored. No changes were observed in proBDNF immunoreactivity. Of interest are findings that proBDNF levels were unchanged by the treatments under study, perhaps reflective of findings that neurons have a ‘limited capacity to process pro-BDNF’ (Matsumoto et al., 2008). Notwithstanding, the current study suggests that BDNF within the hippocampus may play an important role SERT function following a binge exposure to METH. Future studies are needed to demonstrate the potential role of BDNF as a mechanism underlying METH induced tolerance.

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Statement of Interests

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