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Environmental enrichment increases amphetamine-induced glutamate neurotransmission in the nucleus accumbens: a neurochemical study

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Abstract

In addition to dopamine (DA), evidence indicates that glutamatergic regulation of the mesolimbic reward pathway is involved in mediating the abuse-related effects of psychostimulants, including amphetamine. Since rats raised in an enrichment condition (EC) during development are more sensitive to the locomotor stimulant effects of acute amphetamine compared to rats raised in an impoverished condition (IC), the present study examined amphetamine-induced extracellular glutamate and aspartate levels in the nucleus accumbens (NAcc) of EC and IC rats using *in vivo* microdialysis coupled with HPLC-electrochemical detection. Basal extracellular levels of glutamate or aspartate were not significantly different between EC and IC rats. Acute systemic amphetamine (0.5 or 2.0 mg/kg, sc) increased extracellular glutamate levels in NAcc of EC rats (137% or 305% of basal) and IC rats (120% or 187% of basal). Similarly, acute systemic amphetamine (0.5 or 2.0 mg/kg, sc) elevated aspartate levels in NAcc of EC rats (148% or 237% of basal) and IC rats (115% or 170% of basal). Glutamate levels were elevated by amphetamine to a greater extent in EC rats than in IC rats. Pretreatment with systemic MK-801 (0.25 mg/kg, ip), a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist, prevented the acute amphetamine-induced increase in extracellular glutamate and aspartate levels in NAcc. Overall, these results suggest that alterations in glutamate in the NAcc may be involved in the environment-dependent effects of amphetamine.

Keywords

Drug abuse; Environmental enrichment; Amphetamine; Glutamate; Nucleus accumbens; *In vivo* microdialysis

1. Introduction

Evidence indicates that drug abuse is the result of an interaction of genotype and environmental factors (Comings, 1996; McGue et al., 1996). Previous research has shown that animals reared in an enriched condition (EC) relative to an impoverished condition (IC) exhibit greater sensitivity to the acute effects of psychostimulants on mesolimbic dopamine (DA) function and locomotor activity (Bowling et al., 1993; Bowling and Bardo 1994; Bardo et al., 1999).

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Results suggest that environmental enrichment produces profound neuroanatomical and neurochemical changes in various parts of the brain, including neocortex and subcortical structures (Jones and Greenough, 1996; Kempermann et al., 1997; Van Praag et al., 2000). Enrichment has been shown to increase neurogenesis (Nilsson et al., 1999; Segovia et al., 2006), increase dendritic spines on medium size spiny neurons (Comery et al., 1996), increase synaptic density (Rampon et al., 2000) and increase the density of astrocytes (Pham et al., 1999). It is possible that these enrichment-induced modifications are associated with synaptic plasticity within the brain reward circuitry where glutamate neurotransmission plays a modulatory role (Wolf, 1998; Wolf and Xue, 1999; Giorgetti et al., 2001; Melendez et al., 2004).

The nucleus accumbens (NAcc) and its associated limbic structures integrate various neurotransmitter systems, including DA and glutamate inputs (McGinty, 1999; Mogenson et al., 1980; Pennartz et al., 1994). Acute psychostimulants, including amphetamine, have been shown to increase drug-induced extracellular brain monoamines and excitatory amino acids in the NAcc and other limbic structures (Giorgetti et al., 2001; Reid et al., 1997; Shoblock et al., 2003; Xue et al., 1996). Both DA and glutamate are important neurotransmitter systems which could be modulated by environmental manipulations (Pryce et al., 2002).

Environmental enrichment has been found to alter DA function in NAcc in response to psychostimulants (Bardo et al., 1999; Zhu et al., 2004), as well as to alter glutamate function in hippocampus and medial prefrontal cortex (Melendez et al., 2004; Naka et al., 2005). Compared to IC rats, EC rats have reduced subunit expression for *N*-methyl-D-aspartate (NMDA) receptors, but not α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors (Wood et al., 2005), perhaps reflecting a subtype-specific down-regulation in response to an enhanced glutamate signal. However, the role of glutamate release and its regulation in response to psychostimulants in EC and IC rats is not known. Therefore, using in vivo microdialysis, the present study determined basal glutamate and aspartate levels in NAcc from EC and IC rats, as well as acute amphetamine-induced extracellular glutamate and aspartate levels in NAcc. Since NMDA subunit expression is decreased in EC rats (Wood et al., 2005), the effect of the NMDA antagonist MK-801 on amphetamine-induced changes in glutamate levels in EC rats was also determined.

2. Results

Representative probe placements for the rats used in this study are shown in Fig. 1. Overlapping probe placements are not shown in the figure for clarity of presentation.

2.1. Basal extracellular levels of glutamate and aspartate in EC and IC rats

Basal extracellular levels (mean $\pm$ SEM) of glutamate were 220 ± 29 pg/ μ l and 176 ± 44 pg/ μ l in EC and IC rats, respectively. Basal extracellular levels (mean $\pm$ SEM) of aspartate were 17 ± 3 pg/ μ l and 16 ± 2 pg/ μ l in EC and IC rats, respectively. There were no significant basal differences between EC and IC rats in either glutamate or aspartate.

2.2. Acute amphetamine-induced increase in extracellular levels of glutamate in EC and IC rats

As shown in Fig. 2, systemic saline injection did not produce significant changes in basal extracellular glutamate levels for 60 min (Fig. 2, top and bottom panels). However, acute systemic amphetamine (0.5 or 2 mg/kg) increased extracellular glutamate levels to a peak of $\sim 140\%$ and 318% of basal in EC rats (Fig. 2, top and bottom panels). Glutamate levels returned to baseline over time during the post-injection collection period. Separate ANOVAs revealed a significant environmental condition X sample time interaction [$F(19, 110) = 4.84$, $p < 0.05$].

and $F(19, 100) = 6.36, p < 0.05]$ for amphetamine doses of 0.5 and 2 mg/kg, respectively. Subsequent Tukey's HSD tests revealed that the difference between EC and IC rats for both doses was significant during the first two 20-min post-injection sampling time intervals ($p < 0.05$), but not during the later sampling time intervals.

2.3. Acute amphetamine-induced increase in extracellular levels of aspartate in EC and IC rats

Systemic saline injection did not produce significant changes in basal extracellular aspartate levels for 60 min (Fig. 3, top and bottom panels). However, acute systemic amphetamine (0.5 or 2 mg/kg) increased extracellular aspartate levels to a peak of ~148% and 236% of basal in EC rats (Fig. 3, top and bottom panels). Aspartate levels returned to baseline over time during the post-injection collection period. Separate ANOVAs revealed a significant environmental condition X sample time interaction [$F(19, 110) = 4.24, p < 0.05$ and $F(19, 100) = 3.81, p < 0.05]$ for amphetamine doses of 0.5 and 2 mg/kg, respectively. Subsequent Tukey's HSD tests revealed that the difference between EC and IC rats for both doses was significant during the first two 20-min post-injection sampling time intervals ($p < 0.05$), but not during the later sampling time intervals.

2.4. Effect of MK-801 on acute amphetamine-induced increase in extracellular levels of glutamate and aspartate in EC rats

Pretreatment with MK-801 (0.25 mg/kg) prevented the increase in glutamate and in aspartate (Fig. 4) levels induced by acute amphetamine (2 mg/kg) in EC rats. ANOVA revealed no significant effect of sample time for either glutamate or aspartate levels following the combination of MK-801 and amphetamine in EC rats.

3. Discussion

The present study demonstrates that environmental enrichment augments the elevation in glutamate and aspartate levels in NAcc induced by acute amphetamine. Further, the rise in extracellular glutamate and aspartate levels was prevented by pretreatment with MK-801, indicating that NMDA receptors are involved. Our results with MK-801 in blocking the acute amphetamine-induced increase in glutamate and aspartate levels in EC rats are in accord with previous neurochemical studies using standard housed rats (Reid et al., 1997; Wolf and Xue, 1999), as well as with behavioral results showing that MK-801 blocks amphetamine locomotor sensitization in standard housed rats (Karler et al., 1989). The enrichment-induced augmentation in the neurochemical effects observed here is likely related to pharmacodynamic changes in limbic function, rather than to an alteration in amphetamine pharmacokinetics, as previous work indicates that treatment with ^3H -amphetamine produces a similar brain uptake of radiolabel in EC and IC rats (Bardo et al., 1999). It is possible that the enrichment-induced augmentation of glutamate levels in response to amphetamine may explain, at least in part, the exaggerated hyperactivity shown previously in EC rats following an acute dose of amphetamine (Bowling et al., 1993; Bowling and Bardo 1994).

While the source of glutamate release described in the current report was not determined, it is unlikely that extracellular levels reflected primarily synaptic events. Using in vivo microdialysis, basal glutamate levels have been shown previously to be relatively insensitive to calcium and to the sodium channel blocker tetrodotoxin (Timmerman and Westerink, 1997; Xue et al., 1996). In addition, evidence suggests that amphetamine-induced glutamate efflux may involve the formation of reactive oxygen species resulting from metabolic or oxidative stress independent of synaptic activity (Wolf et al., 2000). Future work in this area of investigation may incorporate microsensor technology, which may be more sensitive than microdialysis to neuronally-derived glutamate (Oldenziel et al., 2006).

It is known that the NAcc and related neural pathways are involved in the psychostimulant effects of amphetamine (Wolf, 1998; Rockhold et al., 1998; Wolf et al., 2004; Kalivas and Volkow, 2005). Neuroanatomical studies have indicated that DA neurons in the NAcc receive significant glutamatergic input directly from the limbic cortex and sub-cortical structures (Groenewegen et al. 1980; Kelley and Domesick 1982; Kita and Kitai 1990; Parent and Hazrati, 1995; Sesack et al., 1989; Tzschentke and Schmidt, 2000). Since activation of direct and indirect glutamate pathways increases glutamate release in NAcc following acute amphetamine injection (Robinson and Beart, 1988; Parent and Hazrati, 1995; McGinty, 1999; Vanderschuren and Kalivas, 2000), a potential role for glutamate-mediated mechanisms is implicated in the environment-dependent alteration in amphetamine-induced effects in NAcc.

Regardless of rearing condition, the acute effect of amphetamine on extracellular glutamate and aspartate levels in the current study is consistent with results from previous microdialysis studies using rats raised in standard housing conditions (Reid et al., 1997; Shoblock et al., 2003; Wolf and Xue, 1999; Xue et al., 1996). However, the underlying mechanism for the enrichment-induced enhancement in amphetamine-induced glutamate and aspartate levels remains unclear. Previous reports indicate that environmental enrichment can produce structural, functional and biochemical modifications in neocortex and hippocampus (Jones et al., 1996; Kempermann et al., 1997). Environmental enrichment also has been found to increase neurogenesis, synaptic numbers, spine density, and growth factor expression in cortex and striatum (Comery et al., 1996; Jones et al., 1997; Pham et al., 1999; Segovia et al., 2006). Which one of these factors, if any, underline the enriched-induced increase in amphetamine-induced extracellular glutamate and aspartate levels in NAcc remains to be determined. However, given that the amphetamine-induced increase in glutamate was blocked completely by MK-801 in EC rats, an involvement of NMDA receptors is implicated. Consistent with this, compared to IC rats, EC rats have increased glutamate transmission in cortex and hippocampus (Nichols et al., 2007; Segovia et al., 2006) and decreased expression of NMDA receptor subunits in NAcc (Wood et al., 2005). The decrease in NMDA receptor expression in NAcc may represent a compensatory down-regulation in response to an enhanced glutamate signal. Although the basal levels of glutamate and aspartate did not differ significantly in NAcc in the current report, it is important to note that amino acid transmitter levels were measured when both EC and IC rats were removed from the home cage environments. Thus, it is possible that repeated exposure to novelty during development may have produced phasic increases in glutamate and aspartate in EC rats that were not apparent under the current measurement conditions.

Previous work has shown a difference between NAcc core and shell subregions with regard to basal DA levels (Lecca et al., 2006; Rahman et al., 2007). In the present study, our probe locations for measurement of glutamate and aspartate were primarily in NAcc shell. It is not known if there is a difference between NAcc shell and core subregions in basal extracellular glutamate and aspartate levels, although NMDA receptor subunit expression does not differ between core and shell (Wood et al., 2005). Further studies are needed to determine if there is any core-shell regional difference in glutamate and aspartate levels using the enrichment paradigm.

Glutamate transmission in NAcc has been implicated in sensitization following repeated stimulant use, which may underlie addiction and relapse (Cornish and Kalivas, 2000; Di Ciano et al., 2000; Kalivas and McFarland, 2003; Szumlinski et al., 2000; Wolf, 1998). Although we did not study locomotor activity or behavioral sensitization in relation to glutamate release in NAcc in this report, it is likely that glutamate release has a modulatory role in locomotion via activation of NMDA receptors (Shoblock et al., 2003), as well as via activation of other glutamate receptor subtypes (Giorgetti et al., 2001; Kim and Vezina, 2002). In the current report, the augmented release of glutamate following acute amphetamine in EC rats, which

was blocked completely by MK-801, may explain the exaggerated hyperactivity in response to acute amphetamine noted previously in EC rats (Bowling et al., 1993; Bowling and Bardo 1994). However, since EC rats show less sensitization to repeated amphetamine compared to IC counterparts (Bardo et al., 1995), it is possible that environmental enrichment may also blunt the glutamate-mediated neuroadaptations that occur as a consequence of repeated drug administration, thus protecting against psychostimulant abuse. Consistent with this possibility, EC rats self-administer less amphetamine than IC rats when tested across repeated self-administration sessions (Bardo et al., 2001).

In summary, results from the present study suggest that alterations in glutamate function in NAcc may be involved in the enrichment-induced augmentation in the stimulant effects of acute amphetamine, an effect that depends on activation of brain NMDA receptors. Further work is needed to determine if this enrichment-induced augmentation in excitatory amino acid release following acute amphetamine generalizes to other drug and non-drug reinforcers.

4. Experimental Procedure

4.1. Animals

Male Sprague–Dawley rats were obtained from Harlan Laboratories (Indianapolis, IN) at 21 days of age and were housed with free access to food and water in a colony room in the Division of Laboratory Animal Resources at the University of Kentucky. Rats were maintained on a 12:12 hr light/dark cycle (lights on at 0700 hr). All animal handling procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Kentucky and were performed in accordance with the 1996 version of the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

4.2. Environmental conditions

Upon arrival, rats were assigned randomly to an EC or IC home cage for the duration of the study. EC rats were housed in a large metal cage (60×120×45 cm) with cohorts (8–10 per cage). The environment contained novel hard plastic objects (i.e., commercially available children's toys, PVC pipe, plastic containers, etc.; 14 objects per cage). Rats were removed briefly each day so that 7 of the objects could be replaced with new objects; the remaining objects were rearranged into a novel configuration. IC rats were housed individually in a hanging cage with wire mesh floor and front panel (17×24×20 cm), and solid metal sides, back and top. IC rats were not handled during this rearing period (21–53 days of age). All experiments commenced when rats were 53 days of age under both conditions.

4.3. Drugs

d-Amphetamine sulfate and MK-801 were obtained from Sigma-Aldrich (St. Louis, MO). Drugs were dissolved in 0.9% NaCl (saline) and were injected subcutaneously (sc). Doses represented the salt weight. The doses of amphetamine and MK-801 used in the present study were similar to doses used in previous studies (Shoblock et al., 2003; Xue et al., 1996).

4.4. Surgery

Surgeries for microdialysis were performed as described previously (Rahman et al., 2007) in aseptic conditions under anesthesia induced by xylazine (8 mg/kg, ip) and ketamine hydrochloride (60 mg/kg, ip). Rats were anesthetized and placed in a stereotaxic apparatus (Stoelting, Wood Dale, IL). Microdialysis guide cannulae (18 gauge; Plastics One, Roanoke, VA) were implanted unilaterally in the NAcc according to the atlas of Paxinos and Watson (1986). The cannula was implanted at a 10° angle from the midline using the following coordinates with the incisor bar set at −3.3 mm: AP + 1.7 mm from bregma, L +2.2 mm, and

DV –6.2 mm. To minimize discomfort and pain, rats were given carprofen (5 mg/kg, sc), a non-opioid analgesic, for 3 days after the surgical procedure. Rats were allowed to recover for 3–4 days in their home cages following surgery.

4.5. Microdialysis probe

Loop-style probes were made as described previously (Rahman and McBride, 2002; Rahman et al., 2007) with dialysis membrane (Spectra/Por regenerated cellulose dialysis membrane, molecular weight cutoff of 13,000; Medical Industries, Los Angeles, CA). The outside diameter of the dialysis membrane was 220 μ m. The length of the probe was 2 mm active dialysis membrane. Loop-style probes were used instead of concentric probes because they provide consistent, high basal levels of glutamate, and they sample a large portion of the target area (Rahman and McBride, 2002). All microdialysis probes were inserted 18–24 hrs before the microdialysis procedure (see below).

4.6. In vivo microdialysis procedure

To assess the changes on extracellular glutamate and aspartate levels in NAcc following acute amphetamine, EC and IC rats were assigned randomly to one of 2 groups (n = 8–10 per group): (1) amphetamine (0.5 mg/kg) or (2) amphetamine (2 mg/kg). Rats from each group were treated with injections of saline and amphetamine, separated by 60 min. In a separate study designed to evaluate if blockade of NMDA receptors prevented the effect of amphetamine, EC rats were treated with saline and MK-801 (0.25 mg/kg) separated by 40 min, followed by 20 min later by amphetamine (2 mg/kg).

Microdialysis experiments were performed in clear Plexiglas chambers (25 $\times$ 44 $\times$ 38 cm). Artificial cerebrospinal fluid (ACSF; in mM: 145 NaCl, 2.7 KCl, 1.0 MgCl₂, 1.2 CaCl₂; pH adjusted to 7.3–7.4 with 2 mM sodium phosphate buffer; filtered through a 0.2 μ m sterile filter) was perfused through the probe at a flow rate of 1 μ l/min for 2 hr prior to collection of the baseline samples. After this equilibration period, three baseline samples were collected into polyethylene microfuge tubes every 20 min for an additional 60 min. Five min after the last basal sample collection, rats were given saline, followed 60 min later by amphetamine. For the MK-801 group, EC rats were treated with saline and MK-801 (0.25 mg/kg) separated by 40 min, followed by 20 min later by amphetamine (2 mg/kg). Samples continued to be collected at 20 min intervals for 80 min following the amphetamine injection. Samples were frozen on dry ice and stored at –70°C for HPLC analysis. At the end of the experiments, a 1% bromophenol blue solution was perfused through the probes to verify the placements. Animals were anesthetized deeply with pentobarbital. Brains were removed and fixed for subsequent sectioning to determine the location of the microdialysis probe. Probe placements were evaluated according to the atlas of Paxinos and Watson (1986). Only data from animals with correct probe placements in the NAcc were used in the statistical analyses.

4.7. Quantification of glutamate and aspartate content

Samples were analyzed for glutamate and aspartate levels using high performance liquid chromatography coupled with electrochemical detection (HPLC-ECD, ESA Inc., Chelmsford, MA) as previously described (Melendez et al, 2004). The system consisted of a solvent delivery unit, a Coulochem III electrochemical detector equipped with a 5014B analytical cell and 5020 guard cell. The guard cell was set at +600 mV, electrode 1 at –150 mV, and electrode 2 at +550 mV with the gain at 1 μ A. Precolumn derivatization of glutamate with o-Phthalaldehyde (OPA; Pickering Lab Inc, CA) and 2-Mercaptoethanol (Sigma-Aldrich, MO) was performed using an ESA 542 autosampler. The mobile phase consisted of 100 mM Na₂HPO₄, 22% methanol and 3.5% acetonitrile; pH 6.75 adjusted with phosphoric acid, and pumped through the system at a flow rate of 0.7 μ l/min. Glutamate and aspartate were separated using an analytical column (50mm $\times$ 3mm, Thermo Hypersil-Keystone, PA). The concentration of glutamate and aspartate

in the dialysis samples was quantified by comparing peak heights from samples and external standards using an ESA Chromatography data system (EZChrom Elite, Chelmsford, MA).

4.8. Data analysis

Values were not corrected for in vitro probe recovery efficiency, which was ~15% and in close agreement with published values (Melendez et al., 2004). The baseline was defined as the average glutamate or aspartate level in three samples prior to the saline injection and normalized to 100%. Data were expressed as percent of baseline and were analyzed by one-way ANOVA using SPSS (version 12.0; Chicago, IL). When appropriate, data were further analyzed using *post-hoc* tests (Tukey HSD) for multiple comparisons. The significance level was set at $p < 0.05$.

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Fig. 1. Typical locations of microdialysis probe placements in NAcc as indicated by black lines. Numbers to the right indicate distance in mm from bregma according to Paxinos and Watson (1986). The active probe protruded 2 mm below the distal end of the guide cannulae for NAcc as described in methods.

Fig. 2.

Time course effect of acute amphetamine (AMPH) given at either 0.5 mg/kg (upper panel) or 2 mg/kg (lower panel) on extracellular glutamate level in NAcc of EC and IC rats. After collection of basal samples, rats were given saline (SAL) injection, as indicated by left arrow (↑) and then samples were collected for 60 min before AMPH injection, right arrow (↑). Data represent the mean $\pm$ SEM of 6–7 animals. * $p < 0.05$, significant difference from IC rats at same sampling time interval.

Fig. 3.

Time course effect of acute amphetamine (AMPH) given at either 0.5 mg/kg (upper panel) or 2 mg/kg (lower panel) on extracellular aspartate level in NAcc of EC and IC rats. After collection of basal samples, rats were given saline (SAL) injection, as indicated by left arrow (↑) and then samples were collected for 60 min before AMPH injection, right arrow (↑). Data represent the mean $\pm$ SEM of 6–7 animals. * $p < 0.05$, significant difference from IC rats at same sampling time interval.

Fig. 4.

Time course effect of acute amphetamine (AMPH, 2 mg/kg, sc) given in combination with systemic MK-801 (0.25 mg/kg) on extracellular glutamate or aspartate levels in NAcc of EC rats. After collection of basal samples, rats were given saline (SAL) injection as indicated by left arrow (↑) and samples were collected for 40 min, followed by MK-801, as indicated by middle arrow (↑), and 20 min later, followed by AMPH as indicated by right arrow (↑). Data represent the mean $\pm$ SEM of 6 animals.