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CORTICAL IONOTROPIC GLUTAMATE RECEPTOR ANTAGONISM PROTECTS AGAINST METHAMPHETAMINE-INDUCED STRIATAL NEUROTOXICITY

N. B. GROSS, P. C. DUNCKER AND J. F. MARSHALL*

Department of Neurobiology and Behavior, University of California, Irvine, Irvine, CA, USA

Abstract—Binge administration of the psychostimulant drug, methamphetamine (mAMPH), produces long-lasting structural and functional abnormalities in the striatum. mAMPH binges produce nonexocytotic release of dopamine (DA), and mAMPH-induced activation of excitatory afferent inputs to cortex and striatum is evidenced by elevated extracellular glutamate (GLU) in both regions. The mAMPH-induced increases in DA and GLU neurotransmission are thought to combine to injure striatal DA nerve terminals of mAMPH-exposed brains. Systemic pretreatment with either competitive or noncompetitive *N*-methyl-D-aspartic acid (NMDA) antagonists protects against mAMPH-induced striatal DA terminal damage, but the locus of these antagonists' effects has not been determined. Here, we applied either the NMDA receptor antagonist, (dl)-amino-5-phosphonovaleric acid (AP5), or the alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptor antagonist, dinitroquinoxaline-2,3-dione (DNQX), directly to the dura mater over frontoparietal cortex to assess their effects on mAMPH-induced cortical and striatal immediate-early gene (*c-fos*) expression. In a separate experiment we applied AP5 or DNQX epidurally in the same cortical location of rats during a binge regimen of mAMPH and assessed mAMPH-induced striatal dopamine transporter (DAT) depletions 1 week later. Our results indicate that both ionotropic glutamate receptor antagonists reduced the mAMPH-induced Fos expression in cerebral cortex regions near the site of epidural application and reduced Fos immunoreactivity in striatal regions innervated by the affected cortical regions. Also, epidural application of the same concentration of either antagonist during a binge mAMPH regimen blunted the mAMPH-induced striatal DAT depletions with a topography similar to its effects on Fos expression. These findings demonstrate that mAMPH-induced dopaminergic injury depends upon cortical NMDA and AMPA receptor activation and suggest the involvement of the corticostriatal projections in mAMPH neurotoxicity.

*Corresponding author. Tel: +1-949-824-6636; fax: +1-949-824-2447.
E-mail address: jfmarsha@uci.edu (J. F. Marshall).

Abbreviations: AMPA, alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid; AP5, (dl)-amino-5-phosphonovaleric acid; CG, cingulate cortex; CPu, caudate putamen; DA, dopamine; DAB, diaminobenzidine; DAT, dopamine transporter; DC, dorsocentral; DL, dorsolateral; DM, dorsomedial; DNQX, dinitroquinoxaline-2,3-dione; GLU, glutamate; IEG, immediate-early gene; mAMPH, methamphetamine; Mtx1, primary motor cortex; Mtx2, secondary motor cortex; NAcC, nucleus accumbens core; NAcSh, nucleus accumbens shell; NMDA, *N*-methyl-D-aspartic acid; PBS, phosphate-buffered saline; PrL, prelimbic cortex; SSX, somatosensory cortex; VC, ventrocentral; VL, ventrolateral; VM, ventromedial.

Key words: neurotoxicity, DNQX, AP5, cerebral cortex, striatum, glutamate.

Methamphetamine (mAMPH) is a commonly abused psychostimulant drug that produces long-lasting brain injury and cognitive impairments. Studies of human mAMPH abusers have demonstrated significant neurocognitive deficits that include impairments in executive and psychomotor functions, learning, memory, and attention (Chang et al., 2007; Johanson et al., 2006; Paulus et al., 2003; Volkow et al., 2001). These impairments occur together with structural and functional deficits in the cerebral cortex and striatum. In particular, neuroimaging studies of mAMPH abusers reveal abnormal regional glucose metabolism in the anterior cingulate, orbital frontal, and parietal cortices, as well as reductions of striatal dopamine transporters [DATs; Kim et al. (2005), London et al. (2004), Volkow et al. (2001)]. These human studies are consistent with animal experiments showing persistent reductions in regional cerebral glucose metabolism and long-lasting decreases in several markers of striatal dopaminergic terminal integrity, such as dopamine (DA) content, tyrosine hydroxylase activity, and DAT (Huang et al., 1999; Pontieri et al., 1991; Hotchkiss et al., 1979; Ricaurte et al., 1982).

Acutely, mAMPH administration leads to a leakage of DA from intraneuronal vesicular storage sites into the cytosol, followed by reverse transport of cytosolic DA into the extracellular space via the plasmalemmal uptake carrier, DAT (Schmidt and Gibb, 1985; Sulzer et al., 2005; Fleckenstein et al., 1997). mAMPH-induced elevations in extracellular DA concentration lead to altered activity in striatal efferent pathways. Striatal projection neurons that send efferent fibers to the output nuclei of the basal ganglia influence the cerebral cortex through recurrent circuitry (Gerfen et al., 1990; Alexander and Crutcher, 1990; Parent and Hazrati, 1995). The striatum receives dense glutamatergic input from all regions of cerebral cortex, as well as from intralaminar and ventral thalamic nuclei, via recurrent circuits (McGeorge and Faull, 1989; Mengual et al., 1999; McFarland and Haber, 2001; Smith et al., 2004). The mAMPH-induced increases in striatal DA and glutamate (GLU) neurotransmission are thought to damage DA nerve terminals through the combined influences of oxidative stress and GLU-mediated excitotoxicity (Sonsalla et al., 1991; Nash and Yamamoto, 1992; LaVoie and Hastings, 1999).

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Both the striatum and cerebral cortex are richly populated with glutamatergic synapses (Monaghan et al., 1989; Greenamyre and Young, 1989; Albin et al., 1992), where they contribute to neuronal activation during mAMPH exposure. Ionotropic GLU receptors of the alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and *N*-methyl-D-aspartic acid (NMDA) classes are needed for mAMPH-induced immediate-early gene (IEG) induction within striatal and cerebral cortical neurons (Wang and McGinty, 1996; Gross and Marshall, 2009). Systemic pretreatment with competitive or noncompetitive NMDA antagonists during binge mAMPH administration protects against mAMPH-induced striatal DA terminal damage (Sonsalla et al., 1991; Weihmuller et al., 1992; Stephans and Yamamoto, 1994). Microdialysis studies show that mAMPH-induced increases in striatal extracellular GLU concentrations are necessary for the resulting damage to striatal DA terminals (Stephans and Yamamoto, 1994). It has been argued that increased striatal dopaminergic neurotransmission causes a secondary rise in GLU in the striatum via the recurrent cortico-striato-nigro-thalamo-cortical loop circuit. Direct evidence for the involvement of this circuitry in mAMPH-induced dopaminergic injury comes from Mark et al. (2004), who found that interfering with gamma-amino-butyric acid neurotransmission in the substantia nigra during binge mAMPH administration prevented the mAMPH-induced rise in striatal GLU concentrations as well as subsequent striatal dopaminergic toxicity.

Although the systemic administration of GLU receptor antagonists has provided evidence for involvement of these receptors in mAMPH-induced neurotoxicity, where the antagonists act in the aforementioned circuitry remains to be clarified. The present investigation tested the role of cerebral cortical ionotropic GLU receptors in mAMPH-induced injury to striatal DA terminals. We employed a "cortical well" to administer ionotropic GLU antagonists epidurally, thereby blocking GLU receptors in a region of cortex underneath the well. This blockade was achieved in a minimally invasive manner, by slow diffusion of the antagonist through the cortical layers, with the corpus callosum acting as a diffusional barrier. This epidural application method has been previously used to disinhibit cortical regions, resulting in secondary activation of anatomically restricted populations of striatal neurons (Berretta et al., 1997, 1999; Trevitt et al., 2005). In the first experiment, we demonstrated that frontoparietal epidural application of the NMDA receptor antagonist, AP5, or the AMPA receptor antagonist, dinitroquinoxaline-2,3-dione (DNQX), effectively blocked cortical expression of the IEG, *c-fos*, near the locus of epidural application. We also found that the blockade of GLU neurotransmission in these cortical areas decreased excitatory input to anterior striatum, as assessed by reductions in striatal *c-fos* activation. Subsequently, we evaluated the effects of epidural application of these same GLU receptor antagonists on the DAT depletions arising from a single-day neurotoxic-binge mAMPH regimen. The results of these experiments suggest that cortical NMDA and AMPA receptor activation during mAMPH administration each contributes to augmented glutamatergic neurotransmission at corticostriatal synapses as well as to the consequent mAMPH-induced striatal DAT depletions.

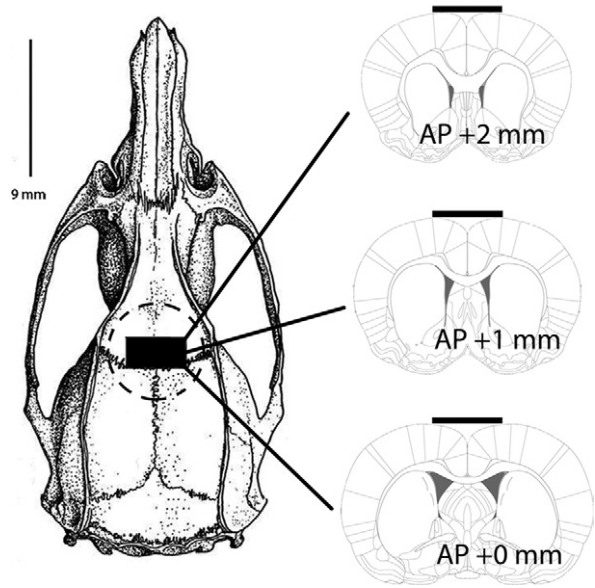

Fig. 1. Schematic diagrams depicting anterior–posterior and medial-lateral extent of cortical well position. Bone diagram (left) illustrating cortical well placement (circular dotted line). A 2 mm×4 mm bone flap (black rectangle), extending anterior +2.0 mm and lateral ±2.0 mm (right), relative to bregma, was removed to expose underlying dura mater. Glutamate antagonists applied in cortical well diffused through skull defect and across dura mater. Schematics derived from Paxinos and Watson (2003).

EXPERIMENTAL PROCEDURES

Subjects

Male Sprague–Dawley rats (275–325 g) were obtained from Charles Rivers Laboratories (Hollister, CA, USA) and individually housed, with food and water *ad libitum*, under a standard 12-h light/dark cycle (lights on 6 AM–6 PM). Animals remained in the holding room for 4 days prior to surgery and were handled every day following surgery. All experiments were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. Approval to conduct the experiments described was obtained from the UC Irvine Institutional Animal Care and Use Committee (IACUC). All efforts were made to minimize the number of animals used and any potential stress and discomfort.

Implantation of cortical well

Rats were anesthetized with Equithesin (10 mM sodium pentobarbital, 256.8 mM chloral hydrate, 86 mM $MgSO_4$, 10.5% propylene glycol, and 12% ethanol, administered at 4.2 mg/kg, i.p.) and placed in a Kopf stereotaxic apparatus. A 2 mm×4 mm bone flap, extending anterior +2.0 mm and lateral ±2.0 mm relative to bregma (Paxinos and Watson, 2003), was removed (Fig. 1). This positioning of the skull defect was chosen because it exposes cingulate, motor, and somatosensory cortical regions to the applied GLU antagonists. Prior research has shown that these cortical regions send strong, topographically organized excitatory projections to the anterior striatum (e.g. McGeorge and Faull,