Neuropharmacology 117 (2017) 328–337

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Sensitizing exposure to amphetamine increases AMPA receptor phosphorylation without increasing cell surface expression in the rat nucleus accumbens

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ARTICLE INFO

Article history:
Received 14 September 2016
Received in revised form
21 December 2016
Accepted 17 February 2017
Available online 20 February 2017

Keywords:
CaMKII
GluA1
GluA2
PKA
PSD
Receptor trafficking

ABSTRACT

Exposure to psychostimulants like cocaine or amphetamine leads to long-lasting sensitization of their behavioral and neurochemical effects. Here we characterized changes in AMPA receptor distribution and phosphorylation state in the rat nucleus accumbens (NAcc) weeks after amphetamine exposure to assess their potential contribution to sensitization by this drug. Using protein cross-linking, biochemical, subcellular fractionation, and slice electrophysiological approaches in the NAcc, we found that, unlike cocaine, previous exposure to amphetamine did not increase cell surface levels of either GluA1 or GluA2 AMPA receptor subunits, redistribution of these subunits to the synaptic or perisynaptic cellular membrane domains, protein-protein associations required to support the accumulation and retention of AMPA receptors in the PSD, or the peak amplitude of AMPA receptor mediated mEPSCs recorded in NAcc slices. On the other hand, exposure to amphetamine significantly slowed mEPSC decay times and increased levels in the PSD of PKA and CaMKII as well as phosphorylation by these kinases of the GluA1 S845 and S831 residues selectively in this cellular compartment. As the latter effects are known to respectively regulate channel open probability and duration as well as conductance, they provide a novel mechanism that could contribute to the long-lasting AMPA receptor dependent expression of sensitization by amphetamine. Rather than increase the number of surface and synaptic AMPA receptors as with cocaine, this mechanism could increase NAcc medium spiny neuron reactivity to glutamate afferents by increasing the phosphorylation state of critical regulatory sites in the AMPA receptor GluA1 subunit in the PSD.

1. Introduction

Repeated exposure to psychostimulant drugs like amphetamine and cocaine enhances their behavioral and neurochemical effects, a form of plasticity termed sensitization. Rats previously exposed to these drugs show enhanced locomotion as well as enhanced dopamine (DA) and glutamate overflow in the nucleus accumbens (NAcc) when subsequently challenged with the drug (Kalivas and Stewart, 1991; Vanderschuren and Kalivas, 2000; Kim et al., 2005). Importantly, sensitization has been linked to addiction vulnerability in humans and animal models (Leyton and Vezina, 2013, 2014). Psychostimulant exposed rats work more and self-administer more drug (Vezina, 2004), an effect consistent with sensitization of the incentive properties of these drugs and the cues associated with them (Robinson and Berridge, 1993, 2008).

Both DA and glutamate act in the NAcc to promote the behavioral expression of psychostimulant sensitization (Vanderschuren and Kalivas, 2000). D1-like DA receptor initiated signaling in NAcc medium spiny neurons (MSNs) activates cAMP-dependent protein kinase (PKA) and Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) (Anderson et al., 2008) both of which phosphorylate a wide array of downstream targets including residues on the

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http://dx.doi.org/10.1016/j.neuropharm.2017.02.018
0028-3908/

GluA1 subunit of the AMPA receptor. This finding is important because it describes one way in which DA and glutamate can interact in the NAcc to drive the expression of sensitization. The serine (S) 845 PKA and S831 CaMKII residues are important regulatory sites in GluA1 that can increase AMPA receptor function and are critical for the expression of LTD, LTP, and the retention of spatial learning (Lee et al., 2003). Phosphorylation at the S845 residue increases channel open probability (Banke et al., 2000) and open duration (Greengard et al., 1991; Han and Whelan, 2009) while phosphorylation at S831 increases channel conductance (Derkach et al., 1999) and together both contribute to the trafficking and synaptic insertion of AMPA receptors (Gao et al., 2006; Derkach et al., 2007). AMPA receptor blockade prevents the expression of locomotor sensitization by amphetamine (Karler et al., 1991; Tzschentke and Schmidt, 1997; Mead and Stephens, 1998; cf, Li et al., 1997) and cocaine (Pierce et al., 1996; Jackson et al., 1998; Bell et al., 2000; cf, Li et al., 1997) and AMPA receptors are functionally upregulated following exposure to either drug (Bell and Kalivas, 1996; Pierce et al., 1996; Suto et al., 2004). Thus, changes in the phosphorylation of the S845 and S831 GluA1 residues are well positioned to influence the expression of sensitization by either amphetamine or cocaine.

It has been reported that sensitizing exposure to cocaine (Boudreau and Wolf, 2005; Boudreau et al., 2007; Kourrich et al., 2007; Ghasemzadeh et al., 2009), but not amphetamine (Nelson et al., 2009), produces a long-lasting increase in cell surface levels of AMPA receptors in the NAcc. The finding with amphetamine is surprising in light of the above evidence supporting a role for cell surface AMPA receptors in the expression of sensitization by either drug. Thus, the present experiments used biochemical, subcellular fractionation, and slice electrophysiological approaches to further characterize the state of AMPA receptors in the NAcc weeks following sensitizing exposure to amphetamine. Our findings confirm that exposure to amphetamine does not increase cell surface expression of AMPA receptors but show that levels of PKA and CaMKII are increased in the PSD as is their phosphorylation of the S845 and S831 residues of GluA1 in this subcellular domain. These findings provide a novel mechanism that could contribute to the long-lasting AMPA receptor dependent expression of sensitization by amphetamine.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats weighing 250–275 g on arrival were obtained from Harlan Sprague-Dawley (Madison, WI) and housed individually in a reverse cycle room (12-h light/12-h dark) with food and water freely available. All procedures were conducted during the dark cycle and according to approved Institutional Animal Care and Use Committee and Institutional Biosafety Committee protocols.

2.2. Exposure to amphetamine and tissue harvest

Rats were administrated five injections of amphetamine (1.5 mg/kg, i.p.) or saline (1.0 ml/kg, i.p.), one injection every other day. In all cases, rats were transported in their housing cages to a distinctive dimly lit experimental room, administered their respective injections, placed back in their housing cages, and returned to the colony room 2 h later. After the last exposure injection, rats were afforded a 2–3 week procedure- and drug-free period after which they were transported to a procedure room, killed by decapitation, their brains removed rapidly, and brain sections and NAcc tissues obtained as described below for cross-linking, subcellular fractionation, immunoprecipitation, immunoblotting, and whole-cell voltage clamp recordings.

2.3. Surface receptor cross-linking

Surface (S) and intracellular (I) levels of the AMPA receptor GluA1 and GluA2 subunits were assessed in the NAcc using the protein cross-linking reagent bis(sulfosuccinimidyl)suberate (BS^3; Pierce Biotechnology) as described previously (Boudreau and Wolf, 2005). Coronal sections (1 mm thick) extending 1.0–3.0 mm from bregma (Paxinos and Watson, 2005) were obtained on ice with a brain matrix. Because similar effects were previously reported in both core and shell subregions of the NAcc (Nelson et al., 2009), in this assay, the entire NAcc (containing both subregions) was bilaterally hand dissected on ice as illustrated in Singer et al. (2009) and chopped into 400 μm slices using a McIllwain tissue chopper (Mickle Laboratory Engineering, Surrey, England). For each animal, all slices were combined, immediately added to an Eppendorf tube containing ice-cold artificial CSF spiked with 2 mM BS^3, and incubated with gentle agitation for 30 min at 4 °C. Cross-linking was terminated by quenching the reaction with 100 mM glycine for 10 min at 4 °C. The slices were then subjected to 2 min of centrifugation at 16000*g*, the supernatant discarded, and the pellets resuspended in ice-cold lysis buffer containing protease and phosphatase inhibitors [(in mM unless stated otherwise) 25 HEPES (pH 7.4); 500 NaCl; 2 EDTA; 1 dithiothreitol; 1 phenylmethanesulfonyl fluoride; 20 NaF; 1 Na orthovanadate; 10 Na pyrophosphate; 1 μM microcystin-LF; 1 μM okadic acid; 1 × protease inhibitor cocktail (EMD Biosciences, San Diego, CA, USA); 0.1% Nonidet P-40 (v/v) (Fluka, Buchs, Switzerland)] and sonicated twice each time for 5 s. Samples were then centrifuged for 5 min at 16000 g and the supernatant fractions aliquoted and stored at −80 °C for subsequent western blot analysis.

2.4. Subcellular fractionation

For all remaining assays, the shell subnucleus of the NAcc was targeted as neurons in this site are known to process the psychomotor activating and incentive motivational properties of psychostimulant drugs (Everitt and Robbins, 2005). Again, 1 mm thick coronal sections extending 1.0–3.0 mm from bregma were cut on ice with a brain matrix and NAcc tissues consisting of the medial and ventral shell were obtained bilaterally with a 2 mm diameter skewed crescent donut punch. All tissues were pooled for each subject and stored at −80 °C until use. Typically, 4 NAcc shell punches were obtained per subject (~5 mg total wet tissue). Subcellular fractions were subsequently prepared as outlined in Fig. 1A. For tissue homogenization and subsequent fractionations, all solutions contained 10 mM Tris (pH = 7.4) as well as protease and phosphatase inhibitors as described above. All centrifugations took place at 4 °C. Briefly, the tissues were transferred to a glass conical tissue grinder and homogenate prepared in 0.2 ml of an ice-cold 320 mM hyperosmotic sucrose buffer containing (in mM) 5 NaF, 1 EDTA (pH = 8.0), 1 PMSF, and 2 Na_3VO_4. After centrifuging the homogenates at 1000 g for 10 min, the supernatant was transferred to a new Eppendorf tube and centrifuged at 10,000 g for 15 min to obtain the cytoplasm fraction and a crude membrane fraction pellet. The latter was resuspended in buffer containing (in mM) 0.5% Triton X-100, 5 NaF, 1 EDTA (pH = 8.0), 1 PMSF, 2 Na_3VO_4 and centrifuged at 10,000 g for 30 min to produce the non-PSD membrane fraction (supernatant) and the pellet. The latter was resuspended in buffer containing (in mM) 1% NP40, 0.1% SDS, 0.5% SDC, 5 NaF, 1 EDTA, and 2 Na_3VO_4 and centrifuged again at 10,000 g for 30 min. The resulting supernatant provided the PSD fraction. Total PSD fraction protein yield ranged from 50 to 100 μg. The cytoplasm,