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Trafficking of calcium-permeable and calcium-impermeable AMPA receptors in nucleus accumbens medium spiny neurons co-cultured with prefrontal cortex neurons

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ABSTRACT

AMPA receptor (AMPAR) transmission onto medium spiny neurons (MSNs) of the adult rat nucleus accumbens (NAc) is normally dominated by GluA2-containing, Ca^{2+}-impermeable AMPAR (CI-AMPARs). However, GluA2-lacking, Ca^{2+}-permeable AMPA receptors (CP-AMPARs) accumulate after prolonged withdrawal from extended-access cocaine self-administration and thereafter their activation is required for the intensified (incubated) cue-induced cocaine craving that characterizes prolonged withdrawal from such regimens. These findings suggest the existence of mechanisms in NAc MSNs that differentially regulate CI-AMPARs and CP-AMPARs. Here, we compared trafficking of GluA1A2 CI-AMPARs and homomeric GluA1 CP-AMPARs using immunocytochemical assays in cultured NAc MSNs plated with prefrontal cortical neurons to restore excitatory inputs. We began by evaluating constitutive internalization of surface receptors and found that this occurs more rapidly for CP-AMPARs. Next, we studied receptor insertion into the membrane; combined with past results, the present findings suggest that activation of protein kinase A accelerates insertion of both CP-AMPARs and CI-AMPARs. We also studied constitutive cycling (net loss of receptors from the membrane under conditions where internalization and recycling are both occurring). Interestingly, although CP-AMPARs exhibit faster constitutive internalization, they cycle at similar rates as CI-AMPARs, suggesting faster reinsertion of CP-AMPARs. In studies of synaptic scaling, long-term (24 h) activity blockade increased surface expression and cycling rates of CI-AMPARs but not CP-AMPARs, whereas long-term increases in activity produced more pronounced scaling down of CI-AMPARs than CP-AMPARs but did not alter receptor cycling. These findings can be used to evaluate and generate hypotheses regarding AMPAR plasticity in the rat NAc following cocaine exposure.

1. Introduction

The nucleus accumbens (NAc), a key player in the reward circuitry of the brain, consists mainly of GABAergic medium spiny neurons (MSNs) (Meredith and Totterdell, 1999). MSNs are critical for motivated behavior, as they serve as an interface between limbic and motor systems (Sesack and Grace, 2010). AMPA-type glutamate receptors (AMPARs) provide the major source of excitatory drive to MSNs, which explains why cocaine-induced plasticity of AMPAR transmission has been linked to robust alterations in cocaine-related behaviors (Wolf and Ferrario, 2010; Wolf and Tseng, 2012).

AMPARs, which are tetramers of GluA1-4 subunits, can be classified based on whether they contain the GluA2 subunit. GluA2-containing receptors are Ca^{2+}-impermeable AMPARs (CI-AMPARs), while those lacking GluA2 are higher-conductance, Ca^{2+}-permeable AMPARs (CP-AMPARs; Isaac et al., 2007; Lee, 2012). CI-AMPARs predominate in NAc synapses of adult rodents under normal circumstances (Conrad et al., 2008; Kourrich et al., 2007; Reimers et al., 2011) and are upregulated following behavioral sensitization to cocaine (Boudreau et al., 2007; Boudreau and Wolf, 2005;

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Kourrich et al., 2007). Different AMPAR plasticity occurs in the 'incubation of drug craving' model, in which rats exhibit progressive intensification (incubation) of cue-induced drug seeking following withdrawal from extended-access drug self-administration (Lu et al., 2004). After prolonged withdrawal from extended-access cocaine self-administration, CP-AMPARs (predominantly homomeric GluA1 receptors) accumulate in excitatory synapses onto NAc MSNs (Conrad et al., 2008). Consistent with their higher conductance, CP-AMPAR accumulation increases the baseline responsiveness of NAc MSNs to excitatory drive (Purgianto et al., 2013), presumably enhancing the response of MSNs to cocaine-related cues. Accordingly, CP-AMPAR activation is required for expression of incubated cue-induced cocaine craving (Conrad et al., 2008; Lee et al., 2013; Loweth et al., 2014; Ma et al., 2014; Mameli et al., 2009; Wolf, 2016). Similar plasticity has recently been demonstrated in the NAc during the incubation of methamphetamine craving (Scheyer et al., 2016). These results suggest that CI-AMPARs and CP-AMPARs can be independently regulated in NAc MSNs in response to psychostimulant treatment. Indeed, subunit composition has been found to influence AMPAR trafficking in other cell types (Esteban et al., 2003; Hanley, 2014; Malinow, 2003; Passafaro et al., 2001; Shi et al., 2001; Xia et al., 2007).

The goal of the present study was to explore potential differences in CI-AMPAR and CP-AMPAR trafficking using an *in vitro* model system comprised of postnatal rat NAc neurons co-cultured with postnatal prefrontal cortex (PFC) neurons from enhanced cyan fluorescent protein (ECFP)-expressing mice. PFC neurons, which innervate NAc MSNs in the intact brain (Sesack et al., 1989), restore excitatory inputs to MSNs in the co-culture but can be distinguished from MSNs based on fluorescence (Sun et al., 2008). MSNs maintained in these co-cultures recapitulate a key aspect of the NAc after incubation of cocaine craving, namely high CP-AMPAR expression (Sun and Wolf, 2009). We have conducted extensive studies of AMPAR trafficking in this co-culture model (Reimers et al., 2014; Sun et al., 2008; Sun and Wolf, 2009; Wolf, 2010b), but have not systematically compared GluA1-containing receptors that include GluA2 (CI-AMPARs) to GluA1-containing receptors that lack GluA2 (CP-AMPARs). Here we used immunocytochemical assays to compare CI-AMPAR and CP-AMPAR trafficking in cultured NAc MSNs.

2. Methods

2.1. Animals

Pregnant Sprague Dawley rats (Harlan, Indianapolis, IN), obtained at 18–21 d of gestation, were housed individually in breeding cages. Postnatal day 1 (P1) pups were decapitated and used to obtain NAc neurons. PFC neurons were obtained from P1 homozygous ECFP-expressing mice [strain B6.129(ICR)-Tg(ACTB-ECFP)1Nagy/J; The Jackson Laboratory, Bar Harbor, ME]. The homozygous ECFP transgenic mouse strain was maintained by mating ECFP male and female mice in-house.

2.2. NAc/PFC co-cultures

As described previously (Sun et al., 2008), the medial PFC of ECFP-expressing P1 mice was dissociated with papain (20–25 U/mL; Worthington Biochemical, Lakewood, NJ) at 37 °C for 30 min. PFC cells were plated at a density of 30,000 cells/well onto coverslips coated with poly-D-lysine (100 μg/mL; Sigma-Aldrich, St. Louis, MO). One to three days later, the NAc from P1 rats was dissociated with papain as above, and plated at a density of 30,000 cells/well with the PFC cells. Co-cultures were grown in Neurobasal medium (Invitrogen, Carlsbad, CA) supplemented with 2 mM GlutaMAX, 0.5% Gentamicin and 2% B27 (Invitrogen), hereafter referred to as "media". One-half of the media was replaced every 3–4 d. Co-cultures were used within 2–3 weeks after plating.

2.3. Drug treatments

Co-cultures were treated with 6-cyano-7-nitroquinoxaline-2,3-dione disodium salt (CNQX) (24 h; 20 μM; Tocris, Bristol, United Kingdom), (−)bicuculline methiodide (24 h; 20 μM; Tocris) or the membrane permeable protein kinase A (PKA) activator Sp-adenosine 3,5-cyclic monophosphorothioate triethylammonium salt (SpcAMPS; 15 min; 10 μM; Sigma-Aldrich) (all drugs dissolved in media). We showed previously that these CNQX and bicuculline treatments elicit synaptic scaling (Reimers et al., 2014; Sun and Wolf, 2009) and that this Sp-cAMPS treatment increases synaptic insertion of GluA1-containing AMPARs (Mangiavacchi and Wolf, 2004a; Sun et al., 2008) in cultured NAc MSNs.

2.4. Surface staining

Surface GluA1 and GluA2 were labeled by incubating live cultures with antibodies recognizing the extracellular N-terminal domains of GluA1 (N-GluA1; 1:10; PC246, aa 271–285; Calbiochem, San Diego, CA) and GluA2 (N-GluA2; 1:20; MAB397, aa 175–430; Millipore, Billerica, MA) in media (37 °C; 15 min). Cells were then fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) (10 min), blocked with 5% donkey serum in PBS (1 h) and incubated with Cy3-conjugated donkey anti-rabbit secondary (1:500; Jackson ImmunoResearch, West Grove, PA) and Alexa 488-conjugated donkey anti-mouse secondary (1:1000; Invitrogen) under non-permeabilized conditions (1 h).

2.5. Receptor cycling

We used a slight modification of a previously described method (Casimiro et al., 2011; Xia et al., 2006, 2007). First, cell surface GluA1 and GluA2 were labeled by incubating live cells with N-GluA1 (1:10) and N-GluA2 (1:20) antibodies in media (37 °C; 15 min). Antibodies were then removed and cells returned to the incubator to allow cycling to occur for 60 min before fixation under non-permeabilizing conditions (4% paraformaldehyde; 10 min). Controls were fixed at 0 min to quantify receptor surface expression prior to cycling. Cells were then blocked and incubated with Cy3- and Alexa 488-conjugated secondary antibodies, as described in Section 2.4, to detect remaining surface receptors.

2.6. Receptor internalization

Surface AMPARs on live neurons were labeled by incubating with N-GluA1 (1:10) and N-GluA2 (1:20) in media for 30 min. To minimize receptor trafficking, this was performed at 15 °C, using a 3% CO_2 refrigerated incubator (Tritech Research, San Diego, CA). Cells were then washed briefly with media to remove primary antibodies and brought to RT (5 or 30 min) to allow internalization of labeled AMPARs (controls were not brought to RT). Next, cells were fixed with 4% paraformaldehyde and incubated with nonconjugated goat anti-rabbit (1:50; Sigma) and goat anti-mouse (1:50; Abcam) secondary antibodies to mask the remaining surface AMPARs (i.e., AMPARs that did not internalize during the RT incubation). Cells were permeabilized (0.1% Triton X-100), blocked and incubated with Cy3-and Alexa 488-conjugated secondary antibodies (1 h) to detect newly internalized GluA1 and GluA2, respectively.