Neuropharmacology 61 (2011) 1141–1151

Contents lists available at ScienceDirect

Neuropharmacology

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

Alterations in AMPA receptor subunits and TARPs in the rat nucleus accumbens related to the formation of Ca^{2+}-permeable AMPA receptors during the incubation of cocaine craving

Carrie R. Ferrario [a,1], Jessica A. Loweth [a], Mike Milovanovic [a], Kerstin A. Ford [a], Gregorio L. Galiñanes [b,2], Li-Jun Heng [b], Kuei Y. Tseng [b], Marina E. Wolf [a,*]

[a] Department of Neuroscience, Rosalind Franklin University of Medicine and Science, 3333 Green Bay Road, North Chicago, IL 60064-3095, USA
[b] Department of Cellular and Molecular Pharmacology, Rosalind Franklin University of Medicine and Science, 3333 Green Bay Road, North Chicago, IL 60064-3095, USA

ARTICLE INFO

Article history:
Received 1 October 2010
Received in revised form
20 December 2010
Accepted 11 January 2011

Keywords:
Cocaine
Ca^{2+}-permeable AMPA receptor
Incubation
Nucleus accumbens
TARP
Subcellular fractionation

ABSTRACT

Cue-induced cocaine seeking intensifies or incubates after withdrawal from extended access cocaine self-administration, a phenomenon termed incubation of cocaine craving. The expression of incubated craving is mediated by Ca^{2+}-permeable AMPA receptors (CP-AMPARs) in the nucleus accumbens (NAc). Thus, CP-AMPARs are a potential target for therapeutic intervention, making it important to understand mechanisms that govern their accumulation. Here we used subcellular fractionation and biotinylation of NAc tissue to examine the abundance and distribution of AMPAR subunits, and GluA1 phosphorylation, in the incubation model. We also studied two transmembrane AMPA receptor regulatory proteins (TARPs), γ-2 and γ-4. Our results, together with earlier findings, suggest that some of the new CP-AMPARs are synaptic. These are probably associated with γ-2, but they are loosely tethered to the PSD. Levels of GluA1 phosphorylated at serine 845 (pS845 GluA1) were significantly increased in biotinylated tissue and in an extrasynaptic membrane-enriched fraction. These results suggest that increased synaptic levels of CP-AMPARs may result in part from an increase in pS845 GluA1 in extrasynaptic membranes, given that S845 phosphorylation primes GluA1-containing AMPARs for synaptic insertion and extrasynaptic AMPARs supply the synapse. Some of the new extrasynaptic CP-AMPARs are likely associated with γ-4, rather than γ-2. The maintenance of CP-AMPARs in NAc synapses during withdrawal is accompanied by activation of CaMKII and ERK2 but not CaMKI. Overall, AMPAR plasticity in the incubation model shares some features with better described forms of synaptic plasticity, although the timing of the phenomenon and the persistence of related neuroadaptations are significantly different.

This article is part of a Special Issue entitled 'Synaptic Plasticity and Addiction'.

1. Introduction

Relapse to cocaine use, even after a long period of abstinence, is a critical problem in treating addiction. Relapse is often triggered by craving elicited by cues or contexts previously associated with cocaine (O'Brien et al., 1992). A number of different models exist for studying cue-induced cocaine craving in rats (e.g., Crombag et al., 2008). We are particularly interested in the incubation model. Incubation refers to the progressive intensification of cue-induced cocaine seeking that occurs after withdrawal from extended access cocaine self-administration. Enhanced drug seeking in this rat model persists >3 months after the last cocaine exposure (Lu et al., 2004). The incubation model is particularly relevant to human cocaine users who undergo prolonged abstinence due to incarceration or hospitalization, and then, once released, encounter previously drug-paired cues that can trigger relapse (Reichel and Bevins, 2009).

Abbreviations: aCSF, artificial cerebral spinal fluid; BS^3, bis(sulfosuccinimidyl) suberate; CaMK, Ca^{2+}/calmodulin-dependent protein kinase; CI-AMPAR and CP-AMPAR, Ca^{2+}-impermeable and Ca^{2+}-permeable AMPAR; DTT, dithiothreitol; EPSC, excitatory postsynaptic current; ERK, extracellular signal-regulated kinase; pS845 GluA1, GluA1 phosphorylated at serine 845; LP1, lysed synaptosomal membranes; NAc, nucleus accumbens; PSD, postsynaptic density; TARP, transmembrane AMPA receptor regulatory protein; WD, withdrawal day.

* Corresponding author. Tel.: +1 847 578 8659; fax: +1 847 578 8515.
E-mail addresses: ferrario@umich.edu (C.R. Ferrario), marina.wolf@rosalindfranklin.edu (M.E. Wolf).
[1] Present address: Pharmacology Department, The University of Michigan, 1000 Wall Street, Ann Arbor, MI 48105, USA.
[2] Present address: Laboratorio de Fisiología de Circuitos Neuronales, Departamento de Fisiología y Biofísica, Facultad de Medicina, Universidad de Buenos Aires, Ciudad de Buenos Aires C1121ABG, Argentina.

0028-3908/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuropharm.2011.01.021

Our studies focus on the role of AMPA-type glutamate receptors (AMPARs) in the incubation of cocaine craving. AMPARs are tetramers composed of GluA1-4 subunits (Shepherd and Huganir, 2007). They are associated with auxiliary subunits, including a family of transmembrane AMPA receptor regulatory proteins (TARPs) that regulate AMPAR trafficking, channel properties, and glutamate affinity (Kato et al., 2010). Medium spiny neurons, the output neurons of the nucleus accumbens (NAc), are excited primarily by AMPARs. This is required for drug seeking in many rat models of cocaine addiction, suggesting AMPAR transmission in the NAc as a key control point for craving and relapse (Kalivas, 2009; Wolf and Ferrario, 2010). Therefore, it is important to compare AMPAR transmission in the NAc of drug-naïve rats and rats that have undergone incubation ("incubated rats").

While most AMPARs in the brain contain the GluA2 subunit, there has been considerable recent interest in AMPARs that lack this subunit; these receptors are Ca^{2+}-permeable (CP-AMPARs) and have higher conductance than GluA2-containing, Ca^{2+}-impermeable AMPARs (CI-AMPARs; Cull-Candy et al., 2006; Isaac et al., 2007; Liu and Zukin, 2007). Our recent studies have shown that CP-AMPARs are expressed at very low levels in NAc synapses of adult drug-naïve rats (Conrad et al., 2008; Reimers et al., 2010), but accumulate in association with the incubation of cue-induced cocaine craving and mediate its expression in rats tested on withdrawal day (WD) 45 (Conrad et al., 2008). The presence of CP-AMPARs in NAc synapses of "incubated rats" was demonstrated using electrophysiological techniques, a bis(sulfosuccinimidyl) suberate (BS^3) protein crosslinking assay showing increased cell surface levels of GluA1 but not GluA2, and co-immunoprecipitation experiments showing an increase in levels of GluA1 not physically associated with other AMPAR subunits (Conrad et al., 2008). Although we first demonstrated CP-AMPARs in the NAc core of "incubated rats", we recently showed that they are also present in the rat NAc shell on WD35-49 (McCutcheon et al., 2010). CP-AMPARs have also been detected in the mouse NAc shell after 35 days of withdrawal from extended access cocaine self-administration (Mameli et al., 2009).

The goal of the present study was to learn more about the subcellular distribution of CP-AMPARs in the NAc of "incubated rats" and to understand how these receptors gain access to NAc synapses and accumulate there during withdrawal. To this end, we used subcellular fractionation and biotinylation to examine the abundance and subcellular distribution of AMPAR subunits, as well as GluA1 phosphorylated at serine 845 (pS845 GluA1), in NAc tissue obtained from control rats or "incubated rats" on WD30-45. We also studied two TARPs, γ-2 and γ-4, that we have previously shown are expressed in the adult rat NAc (Ferrario et al., 2011). No prior studies have evaluated the effect of cocaine (or other drugs of abuse) on TARP expression or distribution. Finally, we measured the activation of several signaling pathways previously linked to AMPAR plasticity.

2. Methods

2.1. Subjects and surgical procedures

All procedures were approved by the Rosalind Franklin University of Medicine and Science Animal Care and Use Committee. Ninety-five Male Sprague Dawley rats (Harlan; 250–275 g) were individually housed (12:12 dark/light). All self-administration sessions were conducted in the dark phase of the cycle with food and water available continually. For catheter implantation, rats were anesthetized with isoflurane gas (Henry Schein, Melville, NY) and given the analgesic banamine (2 mg/kg s.c.) prior to surgery. A silastic catheter (Plastics one, Roanoke, VA) was inserted into the right jugular vein, passed under the skin over the right shoulder and fixed in the mid-scapular region. Rats were given 7 days to recover from surgery prior to beginning self-administration. During this time, catheters were flushed every 1–2 days with sterile saline (0.1 ml, 0.9%).

2.2. Cocaine self-administration

Seven days after surgery, rats began self-administration sessions. Sessions (6 h) were conducted once per day for 10 consecutive days, as in our previous study (Conrad et al., 2008). Self-administration chambers (MED Associates, St. Albans, VT) were located within sound and light attenuating cabinets and were equipped with 2 nose-poke ports located on opposite sides of the chamber, 2 cm above the floor. Responding in one port (active) resulted in the delivery of an intravenous infusion of cocaine (0.5 mg/kg in 32 μl; Coc-SA) or saline (32 μl/infusion; Sal-SA) and the illumination of a cue light inside the port. Responses in the second port (inactive) had no consequences. Infusions were delivered on a fixed ratio 1 schedule with a 10 s time out for the first 10 infusions or first hour (whichever occurred first) followed by a 30 s time out for the remainder of the session. The number of responses in the active and inactive ports and the number of infusions obtained were recorded throughout each session. Additional handled control rats (Han) were treated in an identical manner as self-administration rats, except they were not exposed to the self-administration chambers. Instead, after recovery from catheter implantation surgery, they were brought to holding cages away from their colony room where they remained for 6 h before being returned home. This was repeated on 10 consecutive days. These rats were also flushed with saline before and after being placed in the holding cages. Thus, handled controls rats experienced the same surgery, recovery, time away from the colony and handling as self-administration groups. Comparisons between Coc-SA, Sal-SA and Han groups were made for electrophysiological experiments. Comparisons between Coc-SA and Han groups were used in the lysed synaptosomal membrane (LP1) fraction. For all other subcellular fractionation experiments and biotinylation experiments, comparisons were made between Coc-SA and Sal-SA groups. Sal-SA and Han control groups were used interchangeably because no differences between these groups were found in electrophysiological experiments assessing the presence of CP-AMPARs (see Section 3.2). Furthermore, in biochemical studies, levels of GluA1 and GluA2 proteins in various fractions did not differ between these two groups (data not shown). Similar Han control groups have been used in previous studies (Ferrario et al., 2005; Briand et al., 2008a,b). For biochemical studies, tissue was collected on WD1, WD35 or WD45 following the last self-administration or handling session. Electrophysiological studies were conducted on WD30-33. Details of groups compared, *N* values and withdrawal times are provided below following the detailed methods for each type of experiment.

2.3. Whole-cell patch-clamp recordings

Whole-cell patch-clamp recordings were conducted in the presence of NMDAR (APV) and $GABA_AR$ (picrotoxin) antagonists as previously described (Conrad et al., 2008). Briefly, the rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) before being decapitated. Brains were rapidly removed into ice-cold artificial cerebral spinal fluid (aCSF) containing (in mM): 125 NaCl, 25 $NaHCO_3$, 12.5 glucose, 3.5 KCl, 1.25 NaH_2PO_4, 0.5 $CaCl_2$, 3 $MgCl_2$, 0.05 APV, and 0.05 picrotoxin (pH 7.45, 295–305 mOsm). Coronal slices (300 μm thick) containing the NAc were cut in ice-cold aCSF with a Vibratome, and incubated in warm (~35 °C) aCSF solution constantly oxygenated with 95% O_2–5% CO_2 for at least 60 min before recording. In the recording aCSF (delivered at 2 ml/min), $CaCl_2$ was increased to 2 mM and $MgCl_2$ was decreased to 1 mM. Patch pipettes (6–9 MΩ) were pulled from 1.5 mm borosilicate glass capillaries (WPI, Sarasota, FL) with a horizontal puller (Model P97, Sutter Instrument, Novato, CA), and filled with a solution containing 0.125% Neurobiotin and (in mM): 140 Cs-gluconate, 10 HEPES, 2 $MgCl_2$, 3 Na_2-ATP, 0.3 GTP, 0.1 spermine, 1 QX-314 (pH 7.3, 280–285 mOsm). All chemicals and drugs were purchased from Sigma–Aldrich. Experimental groups used for patch-clamp recording studies: Han ($N = 6$ cells from 3 rats), Sal-SA ($N = 5$ cells from 2 rats), Coc-SA WD30-33 ($N = 5$ cells from 2 rats).

2.4. Tissue collection

One, 35, or 45 days after the last self-administration session (or transport to holding cages for Han group), rats were decapitated and bilateral samples of the NAc (core and shell) were rapidly dissected from a 2 mm coronal section obtained using a brain matrix (Activational Systems, Warren, MI).

2.5. Preparation of LP1 fraction and postsynaptic density fraction (PSD)

The subcellular fractionation method used to obtain these fractions was adapted from Goel et al. (2006). NAc tissue was homogenized (Wheaton Potter-Elvehjem Tissue Grinders, Fisher Scientific, Pittsburgh, PA) in HEPES-buffered sucrose (0.32 M sucrose, 4 mM HEPES, pH 7.4) containing 2 mM EGTA, 50 mM NaF, 10 mM PPi, 1 mM NaOV, 1 μM okadaic acid, 1 mM phenylmethyl sulfonyl fluoride (PMSF), 1 μM microcystin-LF and 1× protease inhibitor cocktail set 1 (Calbiochem, Darmstadt, Germany). The homogenate was centrifuged (800 × g, 10 min, 4 °C) to remove the pelleted nuclear fraction (P1). The resulting supernatant (S1) was centrifuged (10,000 × g, 15 min, 4 °C) to yield a crude membrane fraction (P2) which was washed by resuspending in HEPES-buffered sucrose containing protease and phosphatase inhibitors and centrifuging again. The washed P2 fraction was lysed