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Chronic methamphetamine self-administration dysregulates 5-HT2A and mGlu2 receptor expression in the rat prefrontal and perirhinal cortex: Comparison to chronic phencyclidine and MK-801

Peter U. Hámor[a,b], Jana Šírová[c,d], Tomáš Páleníček[c], Magdalena Zaniewska[e,f], Věra Bubeníková-Valešová[c], Marek Schwendt[a,b,*]

[a] Psychology Department, University of Florida, Gainesville, FL 32611, USA
[b] Center for Addiction Research and Education (CARE) at University of Florida, USA
[c] National Institute of Mental Health, 250 67 Klecany, Czech Republic
[d] 3rd Faculty of Medicine, Charles University, 100 00 Prague 10, Czech Republic
[e] Laboratory of Pharmacology and Brain Biostructure, Institute of Pharmacology, Polish Academy of Sciences, Kraków, PL 31343, Poland
[f] Molecular Biology of Peptide Hormones, Department of Cardiovascular Research, Max-Delbrück-Center for Molecular Medicine, Robert-Rössle-Str. 10, 13125 Berlin, Germany

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ABSTRACT

Chronic methamphetamine (meth) abuse often turns into a compulsive drug-taking disorder accompanied by persistent cognitive deficits and re-occurring psychosis. Possible common neurobiological substrates underlying meth-induced deficits and schizophrenia remain poorly understood. Serotonin 2A (5-HT2A) and metabotropic glutamate 2 (mGlu2) receptors co-regulate psychosis-like behaviors and cognitive function in animals. Therefore, in the present study we examined the effects of chronic exposure to three different drugs known to produce persistent deficits in sensorimotor gating and cognition [meth, phencyclidine (PCP) and MK-801] on the expression of 5-HT2A and mGlu2 within the rat medial prefrontal cortex (mPFC), dorsal hippocampus (dHPC) and perirhinal cortex (PRh). Adult male rats underwent 14 days of: (a) meth self-administration (6 h/day), (b) phencyclidine (PCP; 5 mg/kg, twice/day) administration, or (c) MK-801 (0.3 mg/kg, twice/day) administration. Seven days after the discontinuation of drug administration, tissues of interest were collected for protein expression analysis. We found that despite different pharmacological mechanism of action, chronic meth, PCP, and MK-801 similarly dysregulated 5-HT2A and mGlu2, as indicated by an increase in the 5-HT2A/mGlu2 expression ratio in the mPFC (all three tested drugs), PRh (meth and PCP), and dHPC (MK-801 only). Complementary changes in G-protein expression (increase in $G\alpha_q$ and decrease in $G\alpha_i$) were also observed in the mPFC of meth animals. Finally, we found that 5-HT2A/mGlu2 cooperation can be mediated in part by the formation of the receptor heteromer in some, but not all cortical regions. In summary, these data suggest that a shift towards increased availability (and G-protein coupling) of cortical 5-HT2A vs. mGlu2 receptors may represent a common neurobiological mechanism underlying the emergence of psychosis and cognitive deficits observed in subjects with meth use disorder and schizophrenia.

1. Introduction

Methamphetamine (meth) is the most commonly abused synthetic drug worldwide (United Nations Office on Drugs and Crime, 2016). A significant proportion of meth users develop a pattern of uncontrollable, compulsive drug use that can be classified as meth use disorder (MUD, Paulus et al., 2017). The treatment and recovery of individuals with MUD is complicated by high rates of relapse, and persistent meth-induced psychopathologies. In this respect, memory impairments are among the most pronounced and persistent cognitive complications in MUD (Scott et al., 2007). A number of clinical studies reported deficits in working and episodic memory that can persist weeks or months into the meth-free abstinence (Bechara and Martin, 2004; Gonzalez et al., 2007; Morgan et al., 2012; Simon et al., 2004; Woods et al., 2005). Another pervasive complication in MUD that affects almost 40% of meth users is the emergence of psychotic symptoms

* Corresponding author at: Psychology Department, University of Florida, 114 Psychology Building, 945 Center Drive, Gainesville, FL 32611-2250, USA.
E-mail address: schwendt@ufl.edu (M. Schwendt).

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(Grant et al., 2012). Psychotic episodes can be triggered acutely by recent meth use, or spontaneously re-appear even after prolonged periods of abstinence (Glasner-Edwards and Mooney, 2014; McKetin et al., 2006; Zweben et al., 2004). While meth can precipitate psychotic symptoms in individuals with no history of a primary psychotic disorder, it can also exacerbate psychotic symptoms in schizophrenia patients, hinting at potential commonalities in the underlying neurobiology of these two conditions (Chen et al., 2003; McKetin et al., 2006). Progress in identifying neurobiological substrates underlying cognitive complications and psychosis in MUD is critical, as they can collectively contribute to poor treatment outcomes in MUD (Paulus et al., 2017).

A well-accepted animal model that recapitulates many complications of MUD is the self-administration paradigm, in which animals are allowed to have extended access to self-administered meth for a period of two or more weeks. Specifically, studies by our lab and others have shown that extended-access meth self-administration results in: escalated meth use (Rogers et al., 2008), increased reinstatement of meth-seeking (Rogers et al., 2008; Schwendt et al., 2009), reduced cognitive flexibility (Cox et al., 2016; Parsegian et al., 2011), as well as impaired episodic (object recognition) memory (Reichel et al., 2012; Schwendt et al., 2011) and working memory (Recinto et al., 2012). Extended access to self-administered meth also produces deficits in sensorimotor gating akin to deficits observed in schizophrenia patients (Hadamitzky et al., 2011), or across various animal models of schizophrenia (Braff and Geyer, 1990).

Both cognitive impairments and psychotic symptoms produced by chronic meth are thought to arise from the dysregulation of several neurotransmitter systems within the fronto-striatal circuit. Targeting aberrant dopamine transmission using D2 receptor antagonists is a standard-approach therapy of schizophrenia and more recently, also meth psychosis (Samiei et al., 2016; Verachai et al., 2014). However, D2 receptor antagonists do not typically improve cognitive dysfunction, and in some cases can even impair learning and memory performance in humans (Lustig and Meck, 2005; Saeedi et al., 2006; Xu, 2017) and animals (Abdel-Salam et al., 2012; Hou et al., 2006; Hutchings et al., 2013). Alternatively, recent evidence suggests that simultaneous targeting of serotonin and glutamate neurotransmission, namely 5-HT2A and mGlu2(3) receptors, might produce an anti-psychotic effect comparable to that of D2 receptor antagonists (González-Maeso et al., 2008; Moreno et al., 2016). The validity of this approach is based on findings that demonstrate the: *a)* possible association of mGlu2(3) and 5-HT2A gene polymorphisms with schizophrenia and/or meth-induced psychosis (e.g. Harrison et al., 2008; Tsunoka et al., 2010), *b)* synergistic antipsychotic properties of 5-HT2A antagonists and mGlu2/3 receptor agonists, *c)* co-localization of both receptors across several cortical brain regions, and *d)* close physical (heteroreceptor complex) and functional (signaling cross-talk) interactions between 5-HT2A and mGlu2 receptors (for review see: Wischhof and Koch, 2016). Moreover, targeting 5-HT2A and/or mGlu2 receptors has been shown to improve cognitive function across a variety of psychopathologies, including MUD and schizophrenia (Wischhof and Koch, 2016; Zhang and Stackman, 2015). As such, enhancing mGlu2 receptor function, or inhibiting 5-HT2A receptor activity ameliorated cognitive deficits in pharmacological models of schizophrenia based on NMDA receptor inhibition by drugs like MK-801 (also known as dizocilpine), or phencyclidine (PCP; Griebel et al., 2016; Snigdha et al., 2010). Finally, our data demonstrate that administration of mGlu2 positive allosteric modulator (PAM) reverses object recognition memory deficits in rats with a history of chronic meth self-administration (Schwendt et al., 2011).

While the pharmacological and gene-linkage studies suggest that the mGlu2 and 5-HT2A receptors play a role in manifestation of common MUD and schizophrenia symptoms, (co)regulation of these receptors in either psychopathology is inadequately understood. Postmortem analysis of brain tissue from schizophrenia patients provided contradictory evidence regarding the changes in mGlu2(3) and/or 5-HT2A expression in the forebrain (Ghose et al., 2008; González-Maeso et al., 2008; Muguruza et al., 2016; Rasmussen et al., 2010), while changes in either receptor expression have not been evaluated in MUD. Only a single study analyzed co-regulation of mGlu2 and 5-HT2A following repeated non-contingent administration of meth in mice, showing increased 5-HT2A and decreased mGlu2 receptor expression in the mPFC (Chiu et al., 2014). Despite similarities in the clinical manifestation of MUD and schizophrenia, to this date no studies have concurrently evaluated mGlu2 and 5-HT2A receptor expression in commonly used animal models of these two psychopathologies. The current study aimed to address this knowledge gap by *first*, validating the tools (antibodies) suitable for the detection of mGlu2 and 5-HT2A proteins in rodent brain tissue, *second*, we evaluated the possibility that functional cross-talk occurs via direct physical interaction between these two receptors in the rat brain tissue, and *third*, we analyzed protein expression of said receptors and their partner G-proteins following the chronic exposure to meth or NMDA receptor antagonists (MK-801 and PCP) within the rat brain regions implicated in psychosis-like behavior (Lewis and Sweet, 2009) and episodic (object recognition) memory, as characterized by: (Warburton and Brown, 2015, 2010). *Finally*, we focused on the analysis of possible mGlu2–5-HT2A co-regulation at a withdrawal time (7 days), when many of the post-meth, -PCP and -MK-801 behavioral deficits have been previously observed by our laboratory, as well as others (see above).

2. Materials and methods

2.1. Animals

Male Long-Evans rats (Charles River Laboratories, Wilmington, MA, USA) and male Wistar rats (Velaz, Kolec u Kladna, Czech Republic) weighing 250–300 g at the time of delivery were individually housed in a temperature- and humidity-controlled vivarium on a reversed 12 h light-dark cycle. Rats received ad libitum water and standard rat chow (Harlan-Teklad, Madison, WI, USA) or Velaz, Czech Republic) unless noted otherwise. Rats that were assigned to the self-administration study received 25 g of chow daily (to maintain their weight at ~85% of the free-feeding weight). After the completion of the self-administration regimen, rats were switched to ad libitum food access. Mouse cortical tissue used for the validation of 5-HT2A and mGlu2 antibodies was obtained from adult male *Htr2a*$^{-/-}$ and *GRM2*$^{-/-}$ mice backcrossed for at least 10 generations onto the 129S6/Sv background. Wild-type animals on a 129S6/Sv background were used as controls. All subjects were offspring of heterozygote breeding. For more information see: (González-Maeso et al., 2003; Moreno et al., 2011). Adult male C57BL/6 mice were used to prepare cortical lysates for the 5-HT2A deglycosylation experiment.

All animal procedures were approved by the Institutional Animal Care and Use Committees of the Medical University of South Carolina, or the Expert Committee for Protection of Experimental Animals of the Prague Psychiatric Center and performed in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011) or with the Guidelines of the European Union Council (86/609/EU).

2.2. Drugs

(+)-Methamphetamine hydrochloride (meth) was obtained from Sigma Aldrich (St. Louis, MO, USA), dissolved in saline (0.9% NaCl) to 0.4 mg/ml concentration and used for intravenous self-administration. MK-801 ((5*R*,10*S*)-(+)-5-methyl-10,11-dihydro-5*H* dibenzo [*a*,*d*] cyclohepten-5,10-imine hydrogen maleate; Sigma-Aldrich, Czech Republic), was dissolved in saline and administered intraperitoneally (IP) at 0.3 mg/kg dose (1 ml/kg b.w.). Phencyclidine (1-(1-Phenylcyclohexyl) piperidine; Sigma-Aldrich, Czech Republic) was