Schizophrenia Research xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Schizophrenia Research

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

Changes in the expression of metabotropic glutamate receptor 5 (mGluR5) in a ketamine-based animal model of schizophrenia

Dariusz Zurawek [a,*], Anna Salerno-Kochan [a], Marta Dziedzicka-Wasylewska [a], Agnieszka Nikiforuk [b], Tomasz Kos [b], Piotr Popik [c]

[a] Institute of Pharmacology, Polish Academy of Sciences, Department of Pharmacology, 31-343 Krakow, Smetna Street 12, Poland
[b] Institute of Pharmacology, Polish Academy of Sciences, Department of Behavioural Neuroscience and Drug Development, 31-343 Krakow, Smetna Street 12, Poland
[c] Faculty of Health Sciences, Collegium Medicum, Jagiellonian University, Krakow, Poland

ARTICLE INFO

Article history:
Received 19 December 2016
Received in revised form 30 March 2017
Accepted 7 April 2017
Available online xxxx

Keywords:
Metabotropic glutamate receptor subtype 5
Schizophrenia
Ketamine
Novel object recognition
Autoradiography
In situ hybridization

ABSTRACT

It has been shown that the metabotropic glutamate receptor subtype 5 (mGluR5) is functionally associated with the NMDA subtype of the glutamate receptor family (NMDA receptors). These two receptors colocalize in brain regions associated with schizophrenia. Although the role of the NMDA receptor in cognitive and negative symptoms of schizophrenia is well studied, information about the role of mGluR5 receptors in schizophrenia is sparse. In our work, we show that subchronic administration of ketamine, a well-studied, non-competitive antagonist of NMDA receptors, caused cognitive deficits in rats as shown by testing novel object recognition (NOR). Moreover, we reveal that subchronic administration of ketamine increased the mRNA and protein expression levels of mGluR5 receptors in regions CA1 and CA3 of the dorsal part of the hippocampus, both of which are strongly associated with the formation of visual memory, which is tested via NOR. We postulate that increased expression of mGluR5 receptors in the dorsal part of the hippocampus may reflect compensatory changes to imbalanced glutamate neurotransmission associated with the hypoactivation of NMDA receptors.

1. Introduction

Schizophrenia is a psychiatric disorder connected with positive symptoms including delusions and hallucinations, negative symptoms such as apathy, anhedonia and depression, and deficits in learning and memory that are classified as cognitive disturbances (Lewis and Lieberman, 2000). Although many studies have investigated the molecular changes underlying schizophrenia, the pathophysiology of this mental illness remains poorly understood. It has been widely accepted that schizophrenia is associated with genetic predispositions that, under certain environmental conditions, can lead to the development of this mental illness (Tsuang, 2000). Interactions between genetic and environmental factors result in altered gene expression in the brain of individuals with schizophrenia, manifesting as disturbed signal transduction, monoamine imbalance or even cortical atrophy (Hazlett et al., 2008). Medical use of chlorpromazine since late 50's to relieve psychotic symptoms in patients suffering from schizophrenia (Ban, 2007) together with further observations that chlorpromazine as well as other neuroleptic drugs are dopamine D2 receptor antagonists (Seeman et al., 1975) supported the dopamine hypothesis of schizophrenia.

Although neuroleptics can efficiently diminish positive symptoms, they have negligible effects on cognitive impairments as well as on negative symptoms of schizophrenia (Davis et al., 2003). Negative and cognitive symptoms observed in patients with schizophrenia may be an effect of imbalanced glutamatergic signaling (McAllister et al., 2015; Volk et al., 2015). The glutamatergic theory of schizophrenia is supported by the observation that subchronic administration of ketamine and phencyclidine (PCP), which both belong to the class of the noncompetitive antagonists of the NMDA subtype of the glutamate receptor family, to healthy humans can induce a clinical picture similar to schizophrenia, including the cognitive deficits (Krystal et al., 1994; Adler et al., 1999; Coyle et al., 2012). Moreover, it has been observed that patients witch schizophrenia exhibit exacerbated schizophrenic symptoms when administered a low dose of ketamine (Lahti et al., 2001). It is worth noting that NMDA receptor blockade can modulate activity of dopaminergic neurons in mesolimbic pathway and that dopamine receptor signaling can modulate hippocampal function (Yang et al., 2016). This suggests a close functional connection between dopaminergic and glutamatergic circuits in the brain (Kegeles et al., 2000).

Numerous studies have been performed to understand the role of hypofunction of NMDA receptors in the pathophysiology of schizophrenia (Lewis and Lieberman, 2000). However, the knowledge of the function of metabotropic glutamate receptors (mGluRs) in schizophrenic brain is sparse. Recent research has focused particular attention on

* Corresponding author.
E-mail address: zurawek@if-pan.krakow.pl (D. Zurawek).

http://dx.doi.org/10.1016/j.schres.2017.04.014
0920-9964/

mGluR5 receptors, which have been functionally associated with the NMDA receptor. It has been observed that mGluR5 co-localizes with the NMDA receptor in the striatum and hippocampus which are brain structures involved in the pathophysiology of schizophrenia (Henry et al., 2002; Luccini et al., 2007). Furthermore, both mGluR5 and NMDA receptors are physically linked to each other via scaffold proteins such as SHANK, Homer, GKAP and PSD-95 (Tu et al., 1999). Additionally, mGluR5 plays important roles in the regulation of NMDA receptor function (Attucci et al., 2001; Chen et al., 2011) and in the formation of spatial memory (Lu et al., 1997; Homayoun et al., 2004; Manahan-Vaughan and Braunewell, 2005; Ayala et al., 2009). Thus, it can be postulated that cognitive deficits observed in individuals with schizophrenia may be linked to disturbed expression of mGluR5, especially since it has been observed that cognitive impairments in ketamine models of schizophrenia may be improved by positive allosteric modulators of mGluR5 (Chan et al., 2008; Ayala et al., 2009).

Novel object recognition test (NOR) is an animal paradigm well suited to studying cognitive impairments associated with schizophrenia. NOR is based on the animal's response to a new object introduced into a familiar environment (Ennaceur and Delacour, 1988). It has been shown that subchronic administration of ketamine impairs the natural preference of the animal to explore a new object, a phenomenon related to disturbed memory function (Nikiforuk et al., 2013). Thus, NOR is an etiologically relevant paradigm for studying visual episodic memory in particular (Nikiforuk et al., 2013).

In our present study, our primary aim was to investigate differences in the expression of dopamine D2 and glutamate mGluR5 receptors in rats expressing cognitive deficits, as observed in NOR test, evoked by repeated administration of ketamine in order to find potential molecular markers underlying cognitive impairments observed in schizophrenia.

We used autoradiographic study of radioligand binding to specific receptors as well as in situ hybridization. This approach allowed us to look at the mRNA and protein expression in various brain structures associated with schizophrenia and memory formation.

2. Experimental procedures

2.1. Animals

Male Sprague-Dawley rats (Charles River, Germany) weighted 200–250 g at arrival time. Animals were housed in a temperature of 21 °C(±2 °C), 40–50% humidity colony room under a 12/12 h light/dark cycle (lights on at 06:00 a.m.) with access to food and water ad libitum. During one week of acclimatization period rats were handled at least 3 times. Behavioral testing was performed during the light phase of the light/dark cycle. The experiments were conducted in accordance with European Union guidelines (Directive 2010/63/EU and guidelines 2007/526/EC) and approved by the II Local Ethics Committee for Animal Experiments at the Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland.

2.2. Ketamine administration

Ketamine (115.34 mg/ml of an aqueous solution, Vetoquinol Biowet, Gorzow Wielkopolski, Poland) was injected intraperitoneally at a dose of 30 mg/kg once daily for ten days. Then, the rats underwent a washout period for 10 days during which they did not receive any injections. After the washout period (without any injections), the NOR procedure was started. This ketamine administration paradigm has been shown to induce cognitive impairments in rats as shown by Nikiforuk and Popik (2012).

2.3. Novel object recognition procedure (NOR)

NOR procedure was performed according to previously published experiment (Nikiforuk et al., 2013). At least 1 h before the start of the experiment, rats were acclimatized to the experimental room. Next, animals underwent behavioral test in a dimly lit (25 lx) "open field" and made of dull gray plastic apparatus (66 × 56 × 30 cm). The floor of the apparatus as well as objects were cleaned and dried after each behavioral test. During NOR performance the rats were habituated to the arena (without any objects) for 5 min at 24 h prior to testing. The test session comprised of two 3-min trials separated by an inter-trial interval (ITI) which lasted 1 h. During the familiarization trial (T1), two identical objects (A1 and A2) were presented in opposite corners. The distance from the walls of the open field was approximately 10 cm. During the recognition trial (T2), one of the objects was replaced with a novel object (A = familiar and B = novel). The animals were returned to the home cage after T1. A glass bulb filled with gravel and a plastic bottle filled with sand were the objects used in the test. The heights of the objects were comparable (~12 cm), and both were heavy enough not to be moved by the rats during the test. Half of the animals from each group received the glass bulb as a novel object, and the other half received the plastic bottle. During the recognition trial the location of the novel object was randomly assigned for each tested animal. Exploration of an object was defined by looking, licking, sniffing or touching the object while sniffing, but not leaning against, standing or sitting on the object. The rat was eliminated from the behavioral study if spent <5 s exploring both objects in T1 or T2. The behavior of the rats was recorded using a camera placed above the arena and connected to the Any-maze® tracking system (Stoelting Co., Illinois, USA). An experimenter blinded to the treatment conditions manually assessed the exploration time. Additionally, the distance travelled was automatically measured by the software. Based on the exploration time (E) of the two objects during T2, the discrimination index was calculated as DI = (EB − EA) / (EA + AB).

2.4. Tissue preparation

After completing the NOR test animals were decapitated. The brains were removed from the skull and frozen in a mixture of heptane and dry ice. Then, 12 μm thick coronal brain sections through the cingulate cortex (Cg), striatum (STR), nucleus accumbens septi (NAcc) and dorsal as well as ventral parts of the hippocampus were cut using a Jung CM 3000 cryostat microtome (Leica, Germany). The brain sections were thaw mounted on gelatin-covered slides, air dried and stored at −20 °C until use. For ELISA experiments prefrontal cortex, hippocampus and striatum were dissected from the rat brains, frozen in 2 ml Eppendorf tubes on dry ice and stored at −80 °C until use.

2.5. Dopamine D_2 and glutamate mGluR5 receptor in situ hybridization

In situ hybridization was performed as described by Zurawek et al. (2013) with minor modifications described below. The animal brain sections were fixed in ice-cold 4% formaldehyde. The formaldehyde-fixed brains were shortly washed in phosphate-buffered saline and incubated for 10 min in an ice-cold solution containing 0.25% acetic anhydride and 0.1 M TEA (Sigma Aldrich, USA). Then, tissue sections were dehydrated in ethanol (POCh, Poland) and incubated two times for 10 min in chloroform (POCh, Poland). The prehybridized tissue sections were than washed in decreasing concentrations of ethanol and dried overnight in a stream of air. In further in situ hybridization study we used a mixture of three oligonucleotides complementary to 4–51, 766–813 and 848–901 bp of the coding sequence of the rat mature dopamine D2 receptor mRNA and a mixture of another three oligonucleotides complementary to 1276–1320, 1647–1688 and 1735–1779 bp of the coding sequence of the rat mature glutamate mGluR5 mRNA. Oligonucleotides were labeled at the 3′ end with [35S]dADP (Hartmann Analytic, Germany) using terminal transferase (Fermentas, Lithuania). Then, radiolabeled probes were suspended in a hybridization buffer at a concentration of 1×10^6 disintegration per minute (dpm). Hybridization buffer was prepared in 0.1% DEPC-treated water and included: 4× saline-sodium

Please cite this article as: Zurawek, D., et al., Changes in the expression of metabotropic glutamate receptor 5 (mGluR5) in a ketamine-based animal model of schizophrenia, Schizophr. Res. (2017), http://dx.doi.org/10.1016/j.schres.2017.04.014