Neurobiology of Disease 59 (2013) 126–140

Contents lists available at ScienceDirect

Neurobiology of Disease

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

A "double hit" murine model for schizophrenia shows alterations in the structure and neurochemistry of the medial prefrontal cortex and the hippocampus

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ARTICLE INFO

Article history:
Received 30 January 2013
Revised 19 June 2013
Accepted 17 July 2013
Available online 26 July 2013

Keywords:
Schizophrenia
Animal model
Inhibitory neurotransmission
Adult neurogenesis
Interneuron

ABSTRACT

Both alterations in neurodevelopment and aversive experiences during childhood and adolescence seem important risk factors for schizophrenia. Animal models reproducing these alterations mimic some of the symptoms, constituting a valid approach to study the etiopathology of this disorder. Among these models, the perinatal injection of N-methyl-D-aspartate receptor antagonists and the postweaning social isolation rearing are among the most widely used. Our aim is to combine them in a "double hit" model, which should produce a wider spectrum of alterations. Lister Hooded rats have been subjected to a single injection of MK-801 at postnatal day 7 and socially isolated from postweaning to adulthood. These animals presented increased body weight gain and volume reductions in their medial prefrontal cortex (mPFC) and hippocampus. They also showed an increased number of activated pyramidal neurons and alterations in the numbers of parvalbumin and calbindin expressing interneurons in the mPFC. The expressions of the polysialylated form of the neural cell adhesion molecule and GAD67 are decreased in the mPFC. The mRNA level of *calbindin* was decreased, while that of *calretinin* was increased in the mPFC. The mRNA level of *ERbB4*, a gene associated to schizophrenia, was also altered in this region. All these structural and neurochemical alterations, specially in cortical inhibitory circuits, are similar to those found in schizophrenic patients and are more numerous than in each of the single models. Consequently, the present "double hit" model may be a better tool to study the neurobiological basis of schizophrenia and to explore new therapeutic approaches.

Introduction

Schizophrenia is a highly complex, multifactorial disease, which results in dramatic changes in behavior, perception and cognition. These changes are paralleled by alterations in the structure, neurochemistry and physiology of certain cerebral regions, specially the prefrontal cortex (PFC) and the hippocampus, two regions critically involved in the etiopathology of this psychiatric disorder.

Several structural studies have shown that patients with schizophrenia have lower volumes of PFC and hippocampus than normal control subjects (for review see Levitt et al., 2010; Phillips et al., 2003). Another aspect of structural plasticity, which may be relevant to schizophrenia, given the involvement of the hippocampus in this disorder, is the apparent presence of alterations in adult neurogenesis in the dentate gyrus of schizophrenic patients (Reif et al., 2006).

A closer view to the cerebral cortex of schizophrenic patients has also revealed alterations in neuronal circuitry, specially affecting the structure of neuronal inhibitory networks and their neurotransmission. In fact, current pathophysiological theories of schizophrenia are pointing to the GABAergic system as responsible for some of the alterations in schizophrenic brains (Benes and Berretta, 2001; Lewis and Gonzalez-Burgos, 2008). The inhibitory neurotransmitter GABA and some genes implicated in its metabolism have been associated with schizophrenia (Straub et al., 2007; Zai et al., 2009), specially the 67-kDa isoform of glutamic acid decarboxylase (GAD67) (see Akbarian and Huang, 2006; Beneyto and Lewis, 2011; Curley et al., 2011 for review). These alterations in inhibitory neurotransmission appear to affect particularly certain interneuronal populations, specially those expressing parvalbumin. Decreased density of neurons expressing the phenotypic markers of cortical GABAergic interneurons parvalbumin and calbindin has been found in the PFC (Akbarian et al., 1995; Beasley et al., 2002; Chance et al., 2005; Sakai et al., 2008) and the

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Available online on ScienceDirect (www.sciencedirect.com).

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http://dx.doi.org/10.1016/j.nbd.2013.07.008

hippocampus (Zhang and Reynolds, 2002) in post-mortem studies of subjects with schizophrenia. Interestingly, a recent study suggests that these alterations in inhibitory circuitries may be mediated by *neuregulin 1* (*Nrg1*) and its receptor *ErbB4*, two genes reported as susceptibility loci for schizophrenia, since their signaling controls the development and connectivity of these circuitries in the cerebral cortex (Fazzari et al., 2010).

The structural alterations in cortical inhibitory neurons found in schizophrenia may be mediated by the polysialylated form of the neural cell adhesion molecule (PSA–NCAM), which, through its antiadhesive properties, facilitates neuronal and synaptic remodeling (see Bonfanti, 2006; Rutishauser, 2008 for review), or the partial isolation of neuronal elements (Gomez-Climent et al., 2011). The addition of PSA to NCAM is mediated by the two polysialyltransferases St8SiaII and St8SiaIV (see Hildebrandt et al., 2010 for review). PSA–NCAM is expressed in a subpopulation of interneurons, both in the PFC and the hippocampus of different mammalian species, including humans (Gilabert-Juan et al., 2012; Gomez-Climent et al., 2011; Mikkonen et al., 1998, 1999; Nacher et al., 2002; Varea et al., 2005, 2007b), which have more reduced structural features than those lacking this molecule (Gomez-Climent et al., 2011). Interestingly, both *NCAM* and *ST8SIAII* genes have been associated or suggested to be associated with schizophrenia (Arai et al., 2006; Atz et al., 2007; McAuley et al., 2012; Tao et al., 2007), and alterations in the expression of NCAM and PSA–NCAM have been found in post-mortem studies of this disorder, including some on the hippocampus and the PFC (Barbeau et al., 1995; Brennaman and Maness, 2010; Gilabert-Juan et al., 2012; Sullivan et al., 2007).

To circumvent the intrinsic problems of studying human brains and to explore new experimental therapeutic approaches, several animal models of schizophrenia have been developed during the recent years. Obviously, none of these models mimics completely the disorder, but all of them can reproduce some of its core symptoms. Given the importance of altered neurodevelopment on the etiopathogenesis of schizophrenia, some of these models consist in experimental interventions during embryogenesis or early postnatal development. One of the most used of such models is the administration of N-methyl-D-aspartate (NMDA) receptor antagonists during the perinatal period, which produces certain cognitive and social impairments similar to those found in schizophrenia (Abdul-Monim et al., 2006; Beninger et al., 2002; Hickey et al., 2012; Rung et al., 2005). Perinatal NMDA receptor antagonist administration also reduces GABAergic neurotransmission and the number of parvalbumin expressing neurons in the PFC and the hippocampus in adulthood (Rotaru et al., 2012). The existence of adverse experiences during early-life markedly influences the development of the nervous system and may facilitate, in genetically pre-disposed individuals, the development of psychiatric disorders such as schizophrenia. In this line, it is known that exposing rodents to postweaning social isolation affects brain development and leads to behavioral, morphological and neurochemical alterations during adulthood, which resemble core symptoms of schizophrenic patients (Fone and Porkess, 2008; McLean et al., 2010; Simpson et al., 2010). These alterations include reduced cortical volume (Day-Wilson et al., 2006), as well as deficits in the number of parvalbumin and calbindin interneurons in the hippocampus (Harte et al., 2007).

During the recent years there has been an effort to combine some of the previous animal models of schizophrenia to better reproduce the disorder. A recent report has tested the hypothesis that a "double-hit" model combining MK-801 administration during adulthood and postweaning social isolation rearing of Sprague–Dawley rats, produces greater behavioral and neurochemical effects than either insult alone, with limited results (Hickey et al., 2012). In the present study, we have developed a similar "double hit" model in Lister Hooded rats, in order to find whether the combination of an earlier injection of MK-801, at postnatal day 7 (P7), which may alter different neurodevelopmental processes, and a postweaning social isolation rearing reproduces some of the structural and molecular changes found in the mPFC and the hippocampus of schizophrenic patients, particularly in their inhibitory networks. We have analyzed the volume of these regions, the expression of the immediate early gene *c-Fos* in their pyramidal neurons and the number of proliferating cells and of immature neurons in the hippocampal dentate gyrus. We have also analyzed changes in the number of parvalbumin and calbindin expressing interneurons and the expression of different molecules involved in synaptic/structural plasticity and inhibitory neurotransmission, such as GAD67, synaptophysin, NCAM and PSA–NCAM by means of immunohistochemistry and optical densitometry. Finally, we have quantified and compared the expression of mRNAs for *GAD67*, *synaptophysin*, *NCAM*, *parvalbumin*, *calretinin*, *calbindin*, *ErbB4*, *Nrg1* and the polysialyltransferases genes (*St8SiaII* and *St8SiaIV*) using real-time reverse-transcription polymerase chain reaction (qRT-PCR).

Experimental procedures

Animals

Fifteen pregnant Lister Hooded rats were purchased from Jackson laboratories (Bar Harbor, Maine, USA) and bred in our animal facility. Pregnant rats were housed individually in a controlled temperature room (25 ºC) and on a 12-h light/dark cycle with food and water available ad libitum. After a week, 60 male rats were born from the pregnant rats and were used for the experiments. These animals were assigned randomly to the vehicle or the MK-801 groups. The weight of the rats was determined at postnatal days 7 (P7) and 21 (P21) and after the 8 weeks of isolation (P77), right before their sacrifice. All animal experimentation was conducted in accordance with the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes and was approved by the Committee on Bioethics of the Universitat de València.

Isolation and MK-801 acute treatment

P7 male rat pups were randomly assigned to 2 groups. 28 rats were intraperitoneally injected with a solution of the non-competitive antagonist of the N-methyl-D-aspartate (NMDA) receptor dizocilpine (MK-801, 1 mg/kg, Ascent Scientific, Princeton, USA) and 32 rats were injected with saline solution (0.9% NaCl). All rat pups remained with their mothers until weaning (21 days); at this postnatal day, rats were housed in groups or reared in isolation, thus forming 4 new groups: Socially housed and vehicle injected (Soc-Veh, $n = 15$), socially housed and MK-801 injected (Soc-MK801, $n = 12$), isolated and vehicle injected (Iso-Veh, $n = 17$) and finally, isolated and MK-801 injected (Iso-MK801, $n = 16$). Rats reared in groups were housed 3 per cage ($215 \times 465 \times 145$ mm), while isolated rats were housed in individual cages ($220 \times 220 \times 145$ mm). All rats were housed in the same room, sharing the same controlled light, temperature and humidity. Rats reared in isolation could hear and smell other rats, but were unable to see or have physical contact with them. All animals were handled once a week by the same person, who replaced the bedding of the cage and added food and water. Rats were reared in these conditions during 8 weeks.

Gene expression

A total of 32 rats encompassing the 4 experimental groups were used for qRT-PCR analysis and were sacrificed by decapitation using a guillotine. After this, their brains were removed from the skull and the whole medial prefrontal cortex (mPFC) and hippocampus of each brain were microdissected. Total mRNA was extracted using TriPure reagent (Roche Applied Science, Indianapolis, IN) following manufacturer's instructions. The concentration and purity of total RNA were determined with an Eppendorf BioPhotometer plus (Eppendorf AG, Hamburg, Germany). cDNA synthesis was performed using the Expand Reverse Transcriptase (Roche Applied Science) and oligo-dT primers.