Neuropharmacology xxx (2016) 1–10

Contents lists available at ScienceDirect

Neuropharmacology

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

Relief from detrimental consequences of chronic psychosocial stress in mice deficient for the metabotropic glutamate receptor subtype 7

Daniel Peterlik [a], Christina Stangl [a], Anna Bludau [a], Dominik Grabski [a], Robert Strasser [a], Dominic Schmidt [b], Peter J. Flor [a, *], Nicole Uschold-Schmidt [a, **]

[a] Faculty of Biology and Preclinical Medicine, Laboratory of Molecular and Cellular Neurobiology, University of Regensburg, Regensburg, Germany
[b] Institute of Immunology, University of Regensburg, Regensburg, Germany

ARTICLE INFO

Article history:
Received 13 January 2016
Received in revised form
25 April 2016
Accepted 26 April 2016
Available online xxx

Keywords:
Metabotropic glutamate receptor subtype 7
Knockout
Chronic psychosocial stress
Chronic subordinate colony housing
Affective and somatic pathologies

ABSTRACT

Chronic stress-related psychiatric conditions and comorbid somatic pathologies are an enormous public health concern in modern society. The etiology of these disorders is complex, with stressors holding a chronic and psychosocial component representing the most acknowledged risk factor. During the last decades, research on the metabotropic glutamate receptor (mGlu) system advanced dramatically and much attention was given to the role of the metabotropic glutamate receptor subtype 7 (mGlu7) in acute stress-related behavior and physiology. However, virtually nothing is known about the potential involvement of mGlu7 in chronic psychosocial stress-related conditions. Using the chronic subordinate colony housing (CSC, 19 days) in male mice, we addressed whether central mGlu7 is altered upon chronic psychosocial stressor exposure and whether genetic ablation of mGlu7 interferes with the multitude of chronic stress-induced alterations. CSC exposure resulted in a downregulation of mGlu7 mRNA transcript levels in the prefrontal cortex, a brain region relevant for stress-related behaviors and physiology. Interestingly, mGlu7 deficiency relieved multiple chronic stress-induced alterations including the CSC-induced anxiety-prone phenotype; mGlu7 ablation also ameliorated CSC-induced physiological and immunological consequences such as hypothalamo-pituitary-adrenal (HPA) axis dysfunctions and colonic inflammation, respectively. Together, our findings provide first evidence for the involvement of mGlu7 in a wide range of behavioral and physiological alterations in response to chronic psychosocial stressor exposure. Moreover, the stress-protective phenotype of genetic mGlu7 ablation suggests mGlu7 pharmacological blockade to be a relevant option for the treatment of chronic stress-related emotional and somatic dysfunctions.

1. Introduction

Chronic stress-related medical conditions such as anxiety and depression are an enormous public health concern in modern society (De Kloet et al., 2005). These disorders are often accompanied by somatic comorbidities including cardiovascular diseases (Buckley et al., 2009; Dimsdale, 2008), inflammatory bowel disease (Bernstein et al., 2010; Duffy et al., 1991; Levenstein et al., 2000) as well as chronic pain and infectious diseases (Cohen et al., 1991; Coker et al., 2000; Kiecolt-Glaser et al., 1996). The etiology of these pathologies is complex, with chronic psychosocial stress representing the most acknowledged risk factor (Chrousos, 2009; Heim and Nemeroff, 2001, 1999; Lupien et al., 2009). To date, there is still a dearth of knowledge about the underlying behavioral, physiological, neural, and immunological mechanisms linking chronic stress with such disorders and treatment options are

Abbreviations: ACTH, adrenocorticotropic hormone; ADX71743, 6-(2,4-dimethylphenyl)-2-ethyl-4,5,6,7-tetrahydro-1,3-benzoxazol-4-one; AMN082, *N,N′*-dibenzhydrylethane-1,2-diamine dihydrochloride; ANOVA, analysis of variance; BDNF, brain-derived neurotrophic factor; CA, closed arm; CNS, central nervous system; CORT, corticosterone; CSC, chronic subordinate colony housing; CV, coefficients of variation; EPM, elevated plus-maze; GR, glucocorticoid receptor; HC, hippocampus; HPA, hypothalamo-pituitary-adrenal; HT, hypothalamus; IFN-γ, Interferon-γ; iGlu, ionotropic glutamate receptor; KO, knockout; mesLNC, mesenteric lymph node cell; mGlu, metabotropic glutamate receptor; mGlu7, metabotropic glutamate receptor subtype 7; MMPIP, 6-(4-methoxyphenyl)-5-methyl-3-(pyridin-4-yl)-4H,5H-[1,2]oxazolo[4,5-*c*]pyridin-4-one; NAM, negative allosteric modulator; OA, open arm; PFC, prefrontal cortex; S.E.M., standard error of the mean; SHC, single-housed control; SIH, stress-induced hyperthermia; TA, total arm; VFTD, Venus flytrap domain; WT, wildtype; XAP044, 7-hydroxy-3-(4-iodophenoxy)-4Hchromen-4-one.

* Corresponding author. Faculty of Biology and Preclinical Medicine, University of Regensburg, D-93053 Regensburg, Germany.
** Corresponding author. Faculty of Biology and Preclinical Medicine, University of Regensburg, D-93053 Regensburg, Germany.
E-mail addresses: peter.flor@ur.de (P.J. Flor), nicole.uschold@ur.de (N. Uschold-Schmidt).

http://dx.doi.org/10.1016/j.neuropharm.2016.04.036
0028-3908/

insufficient. Notably, the chronic subordinate colony housing (CSC) paradigm represents a valuable animal model as it mimics the type of health compromising stressors of human daily life through a combination of chronic, psychological, and social aspects of stress. CSC exposure reliably leads to both somatic and affective consequences, including colonic inflammation, stress axes dysfunctions and increased anxiety-related behavior, and thus, represents a powerful model to study the mechanisms underlying various chronic stress-induced pathologies (Langgartner et al., 2015; Reber et al., 2007; Uschold-Schmidt et al., 2012).

The L-glutamatergic system, the primary excitatory neurotransmitter system in the mammalian brain, consists of a diverse family of receptors broadly divided into ionotropic glutamate receptors (iGlus) and metabotropic glutamate receptors (mGlus). In the last decades, many studies focused on the L-glutamate system in the context of acute stress-related behavior and physiology (Kew and Kemp, 2005; Swanson et al., 2005) thereby indicating a clear link to mental illness. The mGlu7 subtype received particular attention. It shows the highest degree of evolutionary conservation and is the most widely distributed mGlu family member in key limbic brain regions associated with anxiety and other stress-related mental conditions (Kinoshita et al., 1998; Masugi et al., 1999). Mainly localized in the presynaptic active zone of glutamate and GABA neurons (Dalezios et al., 2002; Kinoshita et al., 1998; Kosinski et al., 1999), mGlu7 is thought to act as an auto- and heteroreceptor that becomes activated by excessive glutamate release during very high synaptic activity. Mice with genetic ablation of mGlu7 are characterized by deficits in amygdala-dependent fear learning and aversive responses (Fendt et al., 2008; Masugi et al., 1999). Moreover, they display an antidepressant- and anxiolytic-like phenotype in acute behavioral tests (Cryan et al., 2003) as well as alterations in hypothalamo-pituitary-adrenal (HPA) axis functionality including upregulated glucocorticoid receptor- (GR-) dependent feedback suppression and increased hippocampal brain-derived neurotrophic factor (BDNF) protein levels (Mitsukawa et al., 2006). In line with that, partial siRNA-mediated knockdown of mGlu7 also resulted in behavioral alterations including blockade of extinction of conditioned fear and anxiolytic-like effects (Fendt et al., 2008; O'Connor et al., 2013).

With respect to pharmacological manipulation, only a few systemically active allosteric modulators for mGlu7 were identified so far yielding interesting results in acute behavioral paradigms. For instance, the first mGlu7-selective allosteric agonist *N,N′*-dibenzhydrylethane-1,2-diamine dihydrochloride (AMN082) was shown to elevate plasma adrenocorticotropic hormone (ACTH) and corticosterone (CORT) levels (Mitsukawa et al., 2005), displayed mGlu7-dependent antidepressant-like activity (Bradley et al., 2012; Palucha et al., 2007), and modulated acquisition and extinction of conditioned fear (Fendt et al., 2008; O'Connor et al., 2013). Interestingly, besides activating mGlu7, AMN082 was shown to facilitate rapid and lasting mGlu7 internalization – a form of functional antagonism and a possible mechanism for the drugs anxiolytic and antidepressant activity (Pelkey et al., 2007; Peterlik et al., 2016). The two systemically active mGlu7 negative allosteric modulators (NAMs) 6-(4-methoxyphenyl)-5-methyl-3-(pyridin-4-yl)-4H,5H-[1,2]oxazolo[4,5-*c*]pyridin-4-one (MMPIP) and 6-(2,4-dimethylphenyl)-2-ethyl-4,5,6,7-tetrahydro-1,3-benzoxazol-4-one (ADX71743) induced discrepant effects in behavioral tests. MMPIP showed only low anxiolytic activity and reversed the AMN082-induced antidepressant-like effects (O'Connor and Cryan, 2013; Pałucha-Poniewiera and Pilc, 2013), whereas ADX71743 showed robust anxiolytic-like effects in several tests (Kalinichev et al., 2013). Furthermore, the recently discovered first systemically active and mGlu7-selective orthosteric-like antagonist 7-hydroxy-3-(4-iodophenoxy)-4Hchromen-4-one (XAP044), which binds within the Venus flytrap domain (VFTD) of mGlu7, demonstrated a wide spectrum of antistress-, antidepressant-, as well as anxiolytic-like efficacy in acute tests *in vivo* (Gee et al., 2014). Taken together, there is clear evidence for a prominent role of mGlu7 in the modulation of acute stress-related behavior and physiology.

Less attention was given to the role of the L-glutamate system in chronic psychosocial stress in rodents or humans. However, in the last years some data emerged suggesting the L-glutamatergic system also to be a relevant therapeutic target for chronic stress-related emotional disorders (Kendell et al., 2005; Mathews et al., 2012; Sanacora et al., 2012; Yim et al., 2012). Recently, we have described transcript levels of mGlu5 and mGlu7 were affected in the central nervous system (CNS) by chronic psychosocial stressor exposure using the CSC paradigm, suggesting the involvement of the mGlu7 (and mGlu5) subtype in chronic stress-induced somatic and affective pathologies (Peterlik et al., 2016).

The aim of the present study was therefore to investigate in detail the role of mGlu7 in modulating somatic as well as affective consequences induced by chronic male subordination. Here, we focused on genetic ablation of mGlu7 by using knockout (KO) mice in combination with the CSC paradigm and hypothesized that genetic ablation of mGlu7 shapes the vulnerability to chronic psychosocial stress.

2. Material and methods

2.1. Animals

Depending on the experiment, male wildtype C57BL/6 mice (Charles River, Sulzfeld, Germany) or male mGlu7 receptor knockout mice (mGlu7 KO, bred from heterozygous C57BL/6 breeding pairs in the animal facility of the University of Regensburg, Germany) and their male wildtype (WT) littermates, all weighing 19–22 g, were used as experimental mice and individually housed in standard polycarbonate mouse cages (16 × 22 × 14 cm) for one week before the start of CSC procedure. Male CD1 mice (Charles River, Sulzfeld, Germany), weighing 30–35 g, were used as dominants. All mice were kept under standard laboratory conditions (12 h light/dark cycle, lights on at 06:00 a.m., 22 °C, 60% humidity) with free access to tap water and standard mouse diet (ssniff Spezialdiäten GmbH, Soest, Germany). All experimental protocols were approved by the Committee on Animal Health and Care of the local government and conformed to international guidelines on the ethical use of animals. All efforts were made to minimize animals suffering and to reduce number of animals used.

2.2. Experimental design

Experimental mice were either exposed to 19 days of CSC or single-housed for control (SHC) in a genotype- and weight-matched setup.

Experiment 1. To assess the impact of chronic stressor exposure on the metabotropic glutamate receptor system in the CNS, relative mGlu7 transcript levels were assessed in the prefrontal cortex (PFC), hypothalamus (HT) and hippocampus (HC) of C57BL/6 WT SHC and CSC mice. Data represent a pool of two independent experiments.

Experiment 2. To investigate a possible functional role of the mGlu7 receptor in mediating CSC-induced somatic and affective consequences, behavioral, physiological, and immunological parameters typically affected by 19 days of CSC exposure were assessed in mGlu7 KO mice and their WT littermates. In detail, on day 19 of CSC, mice were tested on the EPM between 08:00 and 11:00 a.m. to verify genotype-specific effects of CSC exposure on

Please cite this article in press as: Peterlik, D., et al., Relief from detrimental consequences of chronic psychosocial stress in mice deficient for the metabotropic glutamate receptor subtype 7, Neuropharmacology (2016), http://dx.doi.org/10.1016/j.neuropharm.2016.04.036