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Full-length Article

Blocking metabotropic glutamate receptor subtype 5 relieves maladaptive chronic stress consequences

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ABSTRACT

Etiology and pharmacotherapy of stress-related psychiatric conditions and somatoform disorders are areas of high unmet medical need. Stressors holding chronic plus psychosocial components thereby bear the highest health risk. Although the metabotropic glutamate receptor subtype 5 (mGlu5) is well studied in the context of acute stress-induced behaviors and physiology, virtually nothing is known about its potential involvement in chronic psychosocial stress. Using the mGlu5 negative allosteric modulator CTEP (2-chloro-4-[2-[2,5-dimethyl-1-[4-(trifluoromethoxy)phenyl]imidazol-4yl]ethynyl]pyridine), a close analogue of the clinically active drug basimglurant – but optimized for rodent studies, as well as mGlu5-deficient mice in combination with a mouse model of male subordination (termed CSC, chronic subordinate colony housing), we demonstrate that mGlu5 mediates multiple physiological, immunological, and behavioral consequences of chronic psychosocial stressor exposure. For instance, CTEP dose-dependently relieved hypothalamo-pituitary-adrenal axis dysfunctions, colonic inflammation as well as the CSC-induced increase in innate anxiety; genetic ablation of mGlu5 in mice largely reproduced the stress-protective effects of CTEP and additionally ameliorated CSC-induced physiological anxiety. Interestingly, CSC also induced an upregulation of mGlu5 in the hippocampus, a stress-regulating brain area. Taken together, our findings provide evidence that mGlu5 is an important mediator for a wide range of chronic psychosocial stress-induced alterations and a potentially valuable drug target for the treatment of chronic stress-related pathologies in man.

1. Introduction

Chronic psychosocial stress events are a constant burden within modern societies and strong risk factors for the development of multiple medical conditions (de Kloet et al., 2005). These comprise psychiatric disorders, such as anxiety and depression (de Kloet et al., 2005; McEwen, 2004), which are often accompanied by numerous 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). To date, the detailed behavioral, physiological, neural, and immunological mechanisms linking chronic stress with such disorders are not well understood and therapeutic options are still limited. In this context, the chronic subordinate colony housing (CSC) paradigm serves as a valuable animal model, which mimics the type of health compromising stressors of human daily life with strong face and construct validity, as it combines chronic, psychological, and social aspects of stress. It further causes both somatic and affective symptoms, including colonic inflammation, stress axes dysfunctions and increased anxiety-states. Thus, it represents a powerful tool to

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study the mechanisms underlying various chronic stress-induced pathologies (Langgartner et al., 2015; Reber et al., 2007; Uschold-Schmidt et al., 2012).

Glutamatergic neurotransmission is mediated by ionotropic and metabotropic glutamate receptors (mGlus), and many recent studies suggest that glutamatergic imbalance is key in the molecular pathophysiology of stress-related psychiatric and comorbid somatic disorders (Kendell et al., 2005; Mathews et al., 2012; Peterlik et al., 2016; Sanacora et al., 2012; Yim et al., 2012). Concurrently, glutamatergic approaches for the pharmacotherapy of psychiatric disorders have increasingly received attention. Exploratory clinical trials revealed that a single infusion of a subanesthetic dose of the (2*R*)-2-(methylamino)butanedioic acid (NMDA) receptor antagonist ketamine induced rapid and sustained antidepressant efficacy in severely depressed and treatment-resistant patients (Berman et al., 2000; Zarate et al., 2006). However, the abuse potential and other safety liabilities of ketamine, as well as its cognition-altering and dissociative effects limit its widespread application to the off-label use as emergency intervention in controlled clinical settings. In contrast, therapeutic strategies targeting mGlus may represent a more subtle mode, as they are rather modulatory in nature with the promise to have fewer side-effects than ligand-gated ion channel modulators (Cartmell and Schoepp, 2000; Niswender and Conn, 2010). Particularly the mGlu5 subtype, which is expressed in many brain regions associated with emotional processing and stress-related disorders such as the amygdala, striatum, hippocampus, frontal cortex, and thalamus (Ferraguti and Shigemoto, 2006; Romano et al., 1995), and also present in peripheral tissues (Julio-Pieper et al., 2011; Volpi et al., 2012), has become a recent focus for drug discovery efforts.

Of note, there is physical and functional association of mGlu5 with postsynaptic NMDA receptors, suggesting that mGlu5 negative allosteric modulators (NAMs) could negatively modulate NMDA receptor function (O'Leary et al., 2000). Furthermore, mGlu5 NAMs have been reported to have therapeutic potential for numerous conditions including anxiety disorders (Krystal et al., 2010; Palucha and Pilc, 2007) and gastro-esophageal reflux disease (Keywood et al., 2009). For instance, a recent clinical study revealed the mGlu5-selective allosteric antagonist 2-chloro-4-{[1-(4-fluorophenyl)-2,5-dimethyl-1H-imidazol-4-yl]ethynyl}pyridine (basimglurant) as a promising antidepressant drug with the potential to also ameliorate important comorbidities such as anxiety and pain (Jaeschke et al., 2015; Lindemann et al., 2015). Moreover, 2-chloro-4-[2-[2,5-dimethyl-1-[4-(trifluoromethoxy)phenyl]imidazol-4yl]ethynyl]pyridine (CTEP), another selective NAM at mGlu5, chemically similar to the clinically active antidepressant basimglurant and optimized for its pharmacokinetic properties for the use in rodents, showed promising stress-protective effects under both acute and chronic stress conditions. In detail, oral CTEP was shown to be active in reducing the hyperthermic response in the stress-induced hyperthermia (SIH) test (Lindemann et al., 2011) and also able to alleviate some of the chronic social defeat stress (CSDS)-induced depressive-like symptoms while lacking protective effects on selected physiological parameters (Wagner et al., 2014). However, the stress-protective relevance of mGlu5, especially under permanent chronic psychosocial stress conditions that induce an increased anxiety-state, stress axes dysfunctions, and colonic inflammation, as represented by the CSC paradigm, has not been addressed so far.

Here, we investigated the role of mGlu5 in modulating somatic as well as affective consequences of chronic psychosocial stress induced by chronic male subordination during the CSC paradigm. We hypothesized that genetic and pharmacological inhibition of mGlu5 shape the vulnerability to chronic psychosocial stress. To test this hypothesis, we used conventional mGlu5 knockout (KO) mice in comparison with their wildtype (WT) littermates and also WT mice chronically infused with the mGlu5 NAM CTEP, at different doses, and in combination with the CSC paradigm. Furthermore, we also addressed alterations in mGlu5 mRNA expression and receptor binding in stress-relevant brain regions in response to CSC exposure in WT mice.

2. Material and methods

2.1. Animals

Depending on the experiment, male C57BL/6 WT mice (Charles River, Sulzfeld, Germany) or mGlu5 KO mice and their male WT littermates (bred from heterozygous C57BL/6 breeding pairs in the animal facility of the University of Regensburg, Germany), all weighing 19–22 g, were used as experimental mice and individually housed in standard polycarbonate mouse cages ($16 \times 22 \times 14$ cm) for at least one week before starting the CSC procedure. Male CD1 mice weighing 30–35 g from our own breeding were used as dominants. All mice were kept under standard laboratory conditions (12 h light/dark cycle, lights on at 0600 h, 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.

2.2. Experimental design

Experimental mice (n = 6–24 per housing or treatment group, depending on the number of animals used per experiment and the number of experiments performed/pooled) were either chronically stressed by exposure to the CSC paradigm or single-housed for control (SHC) in a genotype-, treatment- and weight-matched setup. The CSC paradigm lasted for 19 consecutive days and was conducted as described elsewhere (Langgartner et al., 2015; Peterlik et al., 2016; Reber et al., 2007) and in detail below.

Experiment 1: To investigate a possible role for mGlu5 in mediating CSC-induced somatic and affective consequences, mGlu5 KO mice and their WT littermates were exposed to CSC and typical CSC-affected physiological, immunological, and behavioral parameters were assessed. Data represent a pool of two independent experiments.

Experiment 2: To extend the findings obtained in the genetic approach using mGlu5 KO mice, we analyzed typical CSC-affected physiological, immunological, and behavioral parameters in more detail following CSC exposure in C57BL/6 WT mice with pharmacological mGlu5 inhibition. Here, the systemically active mGlu5 NAM CTEP (Lindemann et al., 2011) was applied chronically during CSC exposure via micro-osmotic pumps implanted subcutaneously (*s.c.*, for details see below). Depending on the respective parameter assessed, data represent one single experiment or a pool of up to three independent experiments.

Experiment 3: To verify and to confirm the results of CTEP chronically applied *s.c.*, during CSC exposure, CTEP was administered chronically during CSC via micro-osmotic pumps implanted *s.c.* but attached to an intraperitoneal (*i.p.*) catheter. Data represent one single experiment.

Experiment 4: Finally, to assess the impact of chronic stressor exposure on the central mGlu system, C57BL/6 WT mice were exposed to CSC and relative mRNA transcript levels and receptor binding sites of mGlu5 were assessed in different brain regions,

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