Neurobiology of Stress 2 (2015) 28–33

Contents lists available at ScienceDirect

Neurobiology of Stress

journal homepage: http://www.journals.elsevier.com/neurobiology-of-stress/

Negative allosteric modulation of the mGlu7 receptor reduces visceral hypersensitivity in a stress-sensitive rat strain

Rachel D. Moloney [a, b], Anna V. Golubeva [a], Richard M. O'Connor [c], Mikhail Kalinichev [d], Timothy G. Dinan [a, b], John F. Cryan [a, c, *]

[a] Laboratory of Neurogastroenterology, Alimentary Pharmabiotic Centre, Biosciences Institute, University College Cork, Ireland
[b] Department of Psychiatry, University College Cork, Ireland
[c] Department of Anatomy and Neuroscience, University College Cork, Ireland
[d] Addex Therapeutics SA, Geneva, Switzerland

ARTICLE INFO

Article history:
Received 29 December 2014
Received in revised form
15 March 2015
Accepted 3 April 2015
Available online 4 April 2015

Keywords:
Visceral pain
Anxiety
Wistar-Kyoto
Glutamate

ABSTRACT

Glutamate, the main excitatory neurotransmitter in the central nervous system, exerts its effect through ionotropic and metabotropic receptors. Of these, group III mGlu receptors (mGlu 4, 6, 7, 8) are among the least studied due to a lack of pharmacological tools. mGlu7 receptors, the most highly conserved isoform, are abundantly distributed in the brain, especially in regions, such as the amygdala, known to be crucial for the emotional processing of painful stimuli. Visceral hypersensitivity is a poorly understood phenomenon manifesting as an increased sensitivity to visceral stimuli. Glutamate has long been associated with somatic pain processing leading us to postulate that crossover may exist between these two modalities. Moreover, stress has been shown to exacerbate visceral pain. ADX71743 is a novel, centrally penetrant, negative allosteric modulator of mGlu7 receptors. Thus, we used this tool to explore the possible involvement of this receptor in the mediation of visceral pain in a stress-sensitive model of visceral hypersensitivity, namely the Wistar Kyoto (WKY) rat. ADX71743 reduced visceral hypersensitivity in the WKY rat as exhibited by increased visceral sensitivity threshold with concomitant reductions in total number of pain behaviours. Moreover, AD71743 increased total distance and distance travelled in the inner zone of the open field. These findings show, for what is to our knowledge, the first time, that mGlu7 receptor signalling plays a role in visceral pain processing. Thus, negative modulation of the mGlu7 receptor may be a plausible target for the amelioration of stress-induced visceral pain where there is a large unmet medical need.

1. Introduction

Glutamate signalling has long been implicated in the pathophysiology of pain states (Fundytus, 2001). Visceral pain is among the most poorly understood pain modality characterised by enhanced sensitivity to visceral stimuli. There is a dearth of information regarding the underlying mechanisms of visceral pain, however central sensitisation via excessive glutamatergic signalling has been implicated (Cervero, 2009, 1995). Stress has been shown to be a critical factor in visceral pain pathophysiology, both in terms of increasing the risk to develop visceral hypersensitivity, and an exacerbating or perpetuating factor (Chaloner and Greenwood-Van Meerveld, 2013; Larauche et al., 2012; Johnson and Greenwood-Van Meerveld, 2014; Lee et al., 2015; Prusator and Greenwood-Van Meerveld, 2015; Hong et al., 2015; Theodorou, 2013; Jennings et al., 2014; Olango and Finn, 2014; Aguilera et al., 2013; Mayer et al., 2001b). Functional gastrointestinal disorders (FGIDs) such as irritable bowel syndrome (IBS) are typified by heightened visceral sensitivity as well as altered bowel movements and bloating. Stressful life events both early in life and in adulthood have long been implicated in the pathophysiology of IBS (Mayer, 2000; Mayer et al., 2001a; McEwen, 1998; Fukudo, 2013). Indeed numerous animal models used to investigate the pathogenesis of IBS are stress-based models of visceral hypersensitivity (Larauche et al., 2012; Moloney et al., 2015; Johnson and Greenwood-Van Meerveld, 2014). The potential role of glutamate in the nociceptive signalling of visceral stimuli has recently begun to be appreciated, both at a spinal and supra-spinal level (Lindstrom et al., 2008;

* Corresponding author. Dept Anatomy & Neuroscience, Room 386, Western Gateway Building, University College Cork, Western Rd., Cork, Ireland.
E-mail address: j.cryan@ucc.ie (J.F. Cryan).

http://dx.doi.org/10.1016/j.ynstr.2015.04.001

Crock et al., 2012a; Blackshaw et al., 2011; Cao et al., 2014; Zhou et al., 2014).

Glutamate exerts its effects via ionotropic and metabotropic (mGlu) receptors (Golubeva et al., 2015). Eight mGlu receptors have been identified to date, which are sub-divided according to pharmacology, signal transduction pathways and sequence homology (Cartmell and Schoepp, 2000). mGlu receptors are not only expressed centrally but offer promising pharmacological targets for FGIDs as they are also expressed peripherally (Julio-Pieper et al., 2011). Furthermore, mGlu receptors are now being looked at more intensely as novel therapeutics not only for painful disorders but also psychiatric disorders due to the more attractive safety profile in comparison to their ionotropic counterparts which exhibit psychotomimetic side effects (Newcomer et al., 2000). Recent evidence supports the concept of targeting group 1 metabotropic receptors (mGlu1, mGlu5) as a potential therapeutic approach in animal models of visceral pain (Varty et al., 2005; Neugebauer and Carlton, 2002; Blackshaw et al., 2011; Crock et al., 2012b, 2012a). However, to date evidence for a role of group III receptors, specifically the mGlu7 receptor, is lacking.

From an evolutionary standpoint, mGlu7 receptors are the most highly conserved mGlu isoform (Flor et al., 1997). They are abundantly distributed in the brain, especially in those regions, such as the amygdala, known to be crucial for the emotional processing of painful stimuli (O'Connor et al., 2010). The mGlu7 receptor is of particular interest given that knockout and siRNA studies in mice have indicated altered amygdala-dependent conditioned fear and aversion responses (Callaerts-Vegh et al., 2006; Masugi et al., 1999; Fendt et al., 2008), and reduced anxiety- and stress-related behaviours (Cryan et al., 2003). Moreover, mGlu7 receptor ablation causes dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis and increases hippocampal brain derived neurotrophic factor (BDNF) protein levels (Mitsukawa et al., 2006). More recently, the development of a novel negative allosteric modulator (NAM) of mGlu7 receptors, namely ADX71743 has been identified and characterised showing potential anxiolytic effects *in vivo* (Kalinichev et al., 2013). Taken together, these data may imply a potential role for the mGlu7 receptor in the modulation of visceral hypersensitivity which is comorbid with stress-related psychiatric disorders.

To this end, in the present study, we assessed whether ADX71743 ((+)-6-(2,4-dimethylphenyl)-2-ethyl-6,7-dihydrobenzo[*d*]oxazol-4(5*H*)-one), a potent, selective, and brain-penetrant mGlu7 NAM (Kalinichev et al., 2013) could reduce visceral hypersensitivity in the stress-sensitive Wistar Kyoto (WKY) rat strain. This strain has previously been shown to exhibit visceral hypersensitivity (Gunter et al., 2000; Carroll et al., 2013) in addition to increased anxiety-like behaviours (Hyland et al., 2015). In addition, previous *in situ* hybridization analysis from our lab has revealed that the WKY rats displayed selective increases in mGlu7 receptor mRNA expression in subregions of the hippocampus compared to Sprague Dawley controls (O'Mahony et al., 2010a). Thus, we also investigated whether negative modulation of mGlu7 could ameliorate the anxiogenic profile of this rat strain.

2. Materials and methods

2.1. Animals

Male WKY rats (250–300 g) (Harlan, UK) were used in this study. All animals were group housed in plastic cages (15 × 22 × 9 cm) and were maintained in a temperature controlled room (20 ± 1 °C) with a 12 h light/dark cycle. The animals were allowed one week to acclimatise to the animal facility in University College Cork after arrival. All experiments were conducted in accordance with the European Directive 2010/63/EU and approved by Animal Experimentation Ethics Committee of University College of Cork.

2.2. Experimental design

Two cohorts of animals were used in the current study.

a) **Cohort 1**: ADX71743 or vehicle was administered 30 min prior to colorectal distension (T0). Animals underwent the balloon insertion protocol 10 min later (T10) and allowed to recover until T30. Visceral pain behaviours were assessed at T30 and immediately after, the animals were euthanized.
b) **Cohort 2**: ADX71743 or vehicle was administered 30 min prior to the open field test (T0). 15 min after administration, a blood sample was taken (T15). The animals were introduced into the open field arena at T30. Animals were removed from the arena after 10 min at T40. Repeated blood samples were taken 5 min later (T45), 30 min later (T75) and 15 min later (T90).

2.3. Colorectal distension (CRD)

CRD was performed as previously described (O'Mahony et al., 2010b; O'Mahony et al., 2012). Briefly, animals were fasted overnight (16 h) and on the day of testing, were anaesthetised with isoflurane and a 6 cm latex balloon was inserted into the colorectal cavity, 1 cm from the anus. The animals were allowed to recover for 20 min before CRD commenced. The paradigm used was an ascending phasic distension from 0 mmHg to 80 mmHg over 8 min using a computer-driven electronic barostat (Dual Drive Barostat, Distender Series II, G & J Electronics Inc., Toronto, ON, Canada). The parameters of interest were: (1) the threshold pressure (mmHg) that evokes visually identifiable visceral pain behaviour and (2) the total number of pain behaviours. Postures defined as visceral pain behaviours were abdominal retractions and/or abdominal withdrawal reflex. The treatment groups were randomised and CRD was performed by an experimenter blinded to treatment groups.

2.4. Open field

Animals were allowed to habituate to the testing room 30 min prior to commencing the test. The open field apparatus consisted of a white round arena measuring 90 cm diameter, brightly lit to 1000 lux (Felice et al., 2014). Animals were introduced in to the centre of the arena one at a time and allowed to explore for 10 min. Animals were then removed and placed immediately back in their home cages. The arena was cleaned with 70% ethanol between each trial. Total distance travelled and the distance moved in the inner zone were analysed using a tracking software system (Ethovision, Noldus, The Netherlands). The treatment groups were randomised and the open field test was performed by an experimenter blinded to treatment groups.

2.5. Drug administration

ADX71743 was synthesised at Addex Therapeutics and kindly donated to us. ADX71743 (50, 100, 150 mg/kg) or vehicle (50% water solution of hydroxyl-propyl-β-cyclodextrin (CD)) were administered subcutaneously (s.c.) 30 min prior to commencement of behavioural testing (Kalinichev et al., 2013). The suspensions were homogenised with stainless steel balls for 30 min at 30 Hz in a 2-ml Eppendorf tube, and then vortexed and sonicated for 10 min. All drugs dosed s.c. were administered at 3 ml/kg volume. Suspensions were prepared fresh daily.