Schizophrenia Research 177 (2016) 18–27

Contents lists available at ScienceDirect

Schizophrenia Research

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

Metabotropic glutamate receptor 3 (mGlu3; mGluR3; GRM3) in schizophrenia: Antibody characterisation and a semi-quantitative western blot study

Aintzane García-Bea [a], Mary A. Walker [a], Thomas M. Hyde [b,c,d], Joel E. Kleinman [b], Paul J. Harrison [a,e,1], Tracy A. Lane [a,*,1]

[a] Department of Psychiatry, University of Oxford, Oxford, United Kingdom
[b] Lieber Institute for Brain Development, Baltimore, USA
[c] Department of Neurology, Johns Hopkins School of Medicine, Baltimore, USA
[d] Department of Psychiatry & Behavioral Sciences, Johns Hopkins School of Medicine, Baltimore, USA
[e] Oxford Health NHS Foundation Trust, Warneford Hospital, Oxford, United Kingdom

ARTICLE INFO

Article history:
Received 28 January 2016
Received in revised form 6 April 2016
Accepted 11 April 2016
Available online 27 April 2016

Keywords:
Group II metabotropic glutamate receptor
Methods
Human brain
Psychosis
Antibody validation

ABSTRACT

Background: Metabotropic glutamate receptor 3 (mGlu3, mGluR3), encoded by GRM3, is a risk gene for schizophrenia and a therapeutic target. It is unclear whether expression of the receptor is altered in the disorder or related to GRM3 risk genotype. Antibodies used to date to assess mGlu3 in schizophrenia have not been well validated.

Objective: To characterise six commercially available anti-mGlu3 antibodies for use in human brain, and then conduct a semi-quantitative study of mGlu3 immunoreactivity in schizophrenia.

Methods: Antibodies tested using Grm3$^{-/-}$ and Grm2$^{-/-}$/3$^{-/-}$ mice and transfected HEK293T/17 cells. Western blotting on membrane protein isolated from superior temporal cortex of 70 patients with schizophrenia and 87 healthy comparison subjects, genotyped for GRM3 SNP rs10234440.

Results: One (out of six) anti-mGlu3 antibodies was fully validated, a C-terminal antibody which detected monomeric (~100 kDa) and dimeric (~200 kDa) mGlu3. A second, N-terminal, antibody detected the 200 kDa band but also produced non-specific bands. Using the C-terminal antibody for western blotting in human brain, mGlu3 immunoreactivity was found to decline with age, and was affected by pH and post mortem interval. There were no differences in monomeric or dimeric mGlu3 immunoreactivity in schizophrenia or in relation to GRM3 genotype. The antibody was not suitable for immunohistochemistry.

Interpretation: These data highlight the value of knockout mouse tissue for antibody validation, and the need for careful antibody characterisation. The schizophrenia data show that involvement of GRM3 in the disorder and its genetic risk architecture is not reflected in total membrane mGlu3 immunoreactivity in superior temporal cortex.

1. Introduction

Group II metabotropic glutamate receptors comprise mGlu2 and mGlu3, encoded by GRM2 and GRM3 respectively. They are G protein-coupled receptors, serving primarily as presynaptic autoreceptors, involved in many facets of synaptic plasticity and brain function (Niswender and Conn, 2010). These receptors are implicated in schizophrenia as part of the broader glutamatergic hypotheses of the disorder, in part driven by pharmacological studies showing that group II mGlu agonists can ameliorate deficits caused by NMDA receptor antagonism (Moghaddam and Adams, 1998; for review see Moreno et al., 2009; Moghaddam and Javitt, 2012). This work fostered development of mGlu2/3 agonists as potential antischizophrenia treatments, with a high-profile positive clinical trial for one such drug, pomaglumetad methionil (Patil et al., 2007). Though this finding was not replicated, interest in group II mGluRs in schizophrenia and as antipsychotic drug targets has persisted (Lyon et al., 2011b; Fell et al., 2012; Vinson and Conn, 2012; Lane et al., 2013; Ellaithy et al., 2015; De Filippis et al., 2015; Pritchett et al., 2015; Walker and Conn, 2015), and a recent secondary analysis of the clinical trials suggests that pomaglumetad methionil may have antipsychotic efficacy early in the disease and in patients previously exposed to D2 dopamine antagonists (Kinon et al., 2015).

* Corresponding author at: Department of Psychiatry, University of Oxford, Warneford Hospital, Oxford OX3 7JX, United Kingdom.
E-mail address: tracy.lane@psych.ox.ac.uk (T.A. Lane).
[1] These authors contributed equally.

http://dx.doi.org/10.1016/j.schres.2016.04.015

The pathophysiological role and therapeutic potential of group II mGluRs in schizophrenia is complemented by increasing evidence that GRM3 is a risk gene for the disorder (Harrison et al., 2008). Initially reported in candidate gene studies (Egan et al., 2004), the evidence is now markedly enhanced by the finding that the GRM3 locus is genome-wide significant for schizophrenia (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). Notably, the signal is intragenic, supporting the interpretation that the genetic association is to the gene itself, and that it may operate by altering GRM3 regulation and expression (Sartorius et al., 2008; Kleinman et al., 2011; Harrison, 2015).

As part of the characterisation of GRM3/mGlu3 in schizophrenia, several studies have measured expression of the gene in brain tissue (see Harrison et al., 2008; Hu et al., 2015). Studies of GRM3 mRNA do not show clear differences between schizophrenia cases and controls (Ohnuma et al., 1998; Richardson-Burns et al., 2000; Egan et al., 2004; Bullock et al., 2008; Gonzalez-Maeso et al., 2008; Kim et al., 2012) although there may be a modest increase in prefrontal cortex (Sartorius et al., 2008). The latter authors also reported that a GRM3 risk SNP was associated with decreased expression of a transcript isoform which lacked exon 4 and predicted to encode an mGlu3 variant with a novel C-terminus (Sartorius et al., 2006, 2008). With regard to studies of mGlu3 immunoreactivity, the data are more variable (see Table 1 for summary of existing studies). A major consideration is that most studies have used antibodies which cross react with mGlu2, or antibodies which have not been well characterised to demonstrate their specificity for mGlu3. The one exception is the antibody generated by Corti et al. (2007), which in Grm3$^{-/-}$ mice showed selectivity. With this antibody, they then demonstrated a reduction of mGlu3 dimer in prefrontal cortex in schizophrenia.

Given the renewed, genomically-driven, focus on GRM3, the question of mGlu3 expression in schizophrenia, and its potential modulation by schizophrenia risk genotype, requires a clearer answer than the prior studies permit. Firstly, by using antibodies that have been sufficiently well characterised. To this end, we have tested a range of anti-mGlu3 antibodies, using brain tissue from Grm3$^{-/-}$ mice, and from Grm2$^{-/-}$/3$^{-/-}$ mice, complemented by transfection of cells with human GRM3 cDNA. We also tested a novel antibody directed at the previously-reported novel mGlu3 variant (mGlu3Δ4; Sartorius et al., 2008). Having characterised the antibodies, we used a validated C-terminal anti-mGlu3 antibody for a semi-quantitative immunoblot study of membrane protein from superior temporal cortex from a series of over 150 patients with schizophrenia and comparison subjects, who were genotyped for a GRM3 risk single nucleotide polymorphism (SNP). The superior temporal cortex is implicated in schizophrenia in terms of alterations in volume (Sun et al., 2009), connectivity (Lee et al., 2009), cytoarchitecture (Eastwood and Harrison, 2003; Beasley et al., 2009), and gene expression (Burnet et al., 1996; Eastwood and Harrison, 2005; Schmitt et al., 2011).

2. Materials and methods

2.1. Mouse brain tissue

Brains were taken from adult Grm3$^{-/-}$ knockout mice, Grm2$^{-/-}$/3$^{-/-}$ double knockout mice, and wild-type mice (Lyon et al., 2008, 2011a; De Filippis et al., 2015), snap frozen, and stored at −80 °C. For protein isolation, a small piece of frontal tissue was cut with a clean razor blade, weighed, and homogenised using membrane extraction buffer and a Dounce homogeniser on ice ($n = 3$ adult female mice per genotype).

2.2. Human brain tissue

The demographic details of the human brains used for the main quantitative study are summarised in Table 2 ('Full series'; for explanation of the 'Matched series' see Section 2.9).

The brains were collected at the National Institute for Mental Health (NIMH) and are from a series described and used in previous studies (e.g. Lipska et al., 2006; Eastwood et al., 2010). Briefly, the tissue was obtained with informed consent from the legal next of kin under NIMH protocol no. 90-M-0142. Diagnoses were made by independent reviews of clinical records by two board-certified psychiatrists using DSM-IV criteria. Control subjects were designated as such based on a standardized screening interview with next of kin, in addition to a review of all available medical records and investigators at the medical examiners' offices. Brains were examined macroscopically and microscopically by a board-certified neuropathologist, and all subjects with significant

Table 1
Prior western blot studies of mGlu3 in schizophrenia, showing main methodological features and key findings.

Study	Antibody	Antibody validation	Sample	Sample preparation and method[a]	Brain area[b]	Loading controls	Bands measured	Main findings
Crook et al. (2002)	Anti-mGlu2/3 (Chemicon)	Pre-absorption with mGlu2/3 peptide abolished immunoreactivity.	20 SCZ, 20 CON	Membranes; 7.5% PA gels and 50 μg protein (duplicates).	BA46	Two internal controls in each blot.	~100 kDa	No group difference. Negative correlation with age in controls.
Gupta et al. (2005)	Anti-mGlu2/3 (Upstate Bio-technology)	No data presented; paper refers to earlier papers, but the latter used a Chemicon mGlu2/3 antibody (with peptide pre-absorption used as the control).	16 SCZ, 9 CON	Total homogenates; 5% β-ME and heated at 95 °C for 4 min; 7.5% PA gels and 40 μg protein (duplicates).	BA9, 11, 32 and 46; n. accumbens, putamen, caudate	None stated.	~100–110 kDa	Increase in BA46 in schizophrenia.
Corti et al. (2007)	Anti-mGlu3, raised against residues 16–35 in mouse mGlu3.	Tested using GRM3 cDNA-transfected CHO cells, and grm3$^{-/-}$ mouse brain.	20 SCZ, 35 CON	Membranes; 20 mM DTT, heated at 60 °C for 3 min; 8% PA gels 40 μg protein (triplicates).	BA10	β-actin and Ponceau S. Each gel also contained two internal standards.	~100 and ~200 kDa bands in cells and WT mouse brain; not seen in grm3$^{-/-}$ mouse. Human brain: bands at ~200 kDa and doublet at ~95/100 kDa.	200 kDa band reduced in schizophrenia. Negative correlations with age for 200 kDa and 100 kDa bands.
Ghose et al. (2009)	Anti-mGlu3 (Abcam)	No data presented; the authors refer to a prior paper, but the latter does not provide clarity.	15 SCZ, 15 CON	Total homogenates; 10% PA gels and 20 μg protein (duplicates).	Prefrontal, temporal and motor cortex.	β-tubulin	Monomer; Molecular weight not specified.	mGlu3 decreased in prefrontal cortex in schizophrenia.

[a] DTT: dithiothreitol. ME: mercaptoethanol. PA: polyacrylamide.
[b] BA: Brodmann area. BA9/46: dorsolateral prefrontal cortex. BA10: frontal pole. BA11, BA32: medial prefrontal cortex.