Journal of Affective Disorders 244 (2019) 80–84

Contents lists available at ScienceDirect

Journal of Affective Disorders

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

Short communication

Changes in levels of cortical metabotropic glutamate 2 receptors with gender and suicide but not psychiatric diagnoses

Brian Dean[a,b,*], Clare Duncan[a], Andrew Gibbons[a]

[a] The Molecular Psychiatry Laboratory, The Florey Institute for Neuroscience and Mental Health, Parkville, Victoria, Australia
[b] The Centre for Mental Health, The Faculty of Health, Arts and Design, Swinburne University, Hawthorne, Victoria, Australia.

A B S T R A C T

Background: We previously reported that, compared to controls, there are lower levels of [^{3}H]LY341495 binding to metabotropic 2/3 receptors (GRM2/3) in Brodmann's area (BA) 24, but not 17 or 46, from subjects with major depressive disorders (MDD) but not bipolar disorders (BD) or schizophrenia. To be able to better interpret these data we have now measured levels of GRM2 in two of these cortical regions.

Methods: Using a rabbit anti-metabotropic GRM2 monoclonal antibody with Western blotting we measured levels of GRM2 in BA 24 and 46 from subjects with MDD, BD, schizophrenia and controls (n = 15 per group).

Results: Compared to controls, levels of GRM2, normalised to β-actin, did not differ in BA 24 or 46 from subjects with MDD, BD or schizophrenia (p from 0.36 to 0.79). Levels of GRM2 in BA 46, but not BA 24, were significantly higher in males compared to females ($p < 0.01$) and in suicide completers ($p < 0.01$) compare to death by other causes.

Limitations: Our cohort sizes, whilst being comparable to many postmortem CNS studies, are relatively low.

Conclusions: Our data suggests levels of GRM2 are not altered in two cortical regions from subjects with mood disorders or schizophrenia. Given we have found lower levels of [^{3}H]LY341495 binding to GRM2/3 in BA 24 from subjects with MDD, our new data argues the lower levels of radioligand binding was due to lower levels of GRM3. Our data also suggests that glutamatergic activity through GRM2 in BA 46 may differ with gender and suicide ideation.

1. Introduction

We have reported that, compared to controls, there are lower levels of [^{3}H]LY341495 binding in Brodmann's area (BA) 24, but not BA 17 or 46, from subjects with major depressive disorders (MDD) (McOmish et al., 2016). [^{3}H]LY341495 binding was not altered in those three cortical regions from subjects with bipolar disorders (BD) or schizophrenia. As [^{3}H]LY341495 binds to the mammalian metabotropic glutamate receptors (GRM) 2 and 3 (Johnson et al., 1999; Wright et al., 2001), our data is consistent with the notion that there is a decrease in the total number of GRM2 plus GRM3 in BA 24 from subjects with MDD. Our finding of lower levels of [^{3}H]LY341495 binding in BA 24 from subjects with MDD did not agree with a previous report that levels of [^{3}H]LY341495 binding was not different in BA 24 from subjects with psychotic and non-psychotic depression, BD or schizophrenia (Matosin et al., 2014).

In addition to radioligand binding, a number of studies have measured levels of total GRM2 plus GRM3 protein or GRM2 and GRM3 mRNA in the cortex of subjects with psychiatric disorders. Using antibodies that could not discriminate between GRM2 and GRM3, studies have reported higher levels of total GRM2/3 in BA 10 from subjects with MDD (Feyissa et al., 2010) and either no change (Crook et al., 2002) or higher (Gupta et al., 2005) levels of total GRM2/3 in BA 46 from subjects with schizophrenia. In addition, a study has reported lower levels of GRM3, but not GRM2, mRNA in BA 9 and no changes in levels of GRM2 or GRM3 mRNA in BA 4, 7, 11, 19, 24 and 38 from subjects with schizophrenia (Ghose et al., 2008). Hence, despite radioligand binding, mRNA and protein studies, there is a relative paucity of data specific to levels of cortical GRM2 or GRM3 in the cortex of subjects with psychiatric disorders. In addition, there appears to be no data on individual levels of GRM2 or GRM3 protein in the cortex of subjects with psychiatric disorders.

Given the lack of data on levels of GRM2 and GRM3 protein, and our data on [^{3}H]LY341495 binding, we decided to measure levels of GRM2 in the BA 24 and 46 from subjects with MDD, BD, schizophrenia and a group of age and gender matched controls.

* Corresponding author at: The Molecular Psychiatry Laboratory, The Florey Institute for Neuroscience and Mental Health, 30 Royal Parade, Parkville, Victoria 3052, Australia.
E-mail addresses: brian.dean@florey.edu.au, anddali@unimelb.edu.au (B. Dean).

https://doi.org/10.1016/j.jad.2018.10.088
Received 20 August 2018; Received in revised form 26 September 2018; Accepted 5 October 2018
Available online 06 October 2018
0165-0327/

2. Experimental procedures

2.1. Tissue collection

Consent to collect CNS tissue postmortem was from the Ethics Committee of the Victorian Institute of Forensic Medicine. Tissue collection and processing, case history reviews and diagnostics was as reported previously (McOmish et al., 2016). Hence, case histories were reviewed using the Diagnostic Instrument for Brain studies (Roberts et al., 1998); these data were used by two psychiatrists and a senior psychologist to reach a census diagnoses based on DSM IV criteria. For each case, the left CNS hemisphere was collected, sliced according to a standardised protocol and frozen at −80 °C to minimise autolytic degradation. Tissue was excised from Brodmann's area (BA) 46 and BA24 from subjects with MDD ($n = 15$), BD ($n = 15$), schizophrenia ($n = 15$) and 15 subjects with no history of psychiatric disorders (Controls).

2.2. Western blotting

Crude homogenates were prepared by homogenising tissue from BA 24 and BA 46 into 5% w/v in 10 mM Tris (pH 7.4) containing 1% SDS and 1 mM Na_3VO_4. The protein concentrations in these homogenates was measured using the Bio-Rad DC modified Lowry protein assay adapted for microplates prior to storage at −80 °C.

On the day of electrophoresis, homogenates containing 15 μg protein were boiled for 5 min before being resolved on a 10% polyacrylamide gel. Duplicate samples from a case from each diagnostic group and a matched control were loaded in consecutive lanes on the same gel (Supplementary Fig. 1). Molecular weight standards were resolved in a lane of each gel.

Proteins were transferred on to nitrocellulose membranes (confirmed by staining each membrane with 0.2% Ponceau S in 3% trichloroacetic acid), each membrane was blocked with 5% non-fat milk powder in 1X TTBS for 1hr at RT and then incubated overnight at 4 °C with rabbit anti-metabotropic GRM2 monoclonal antibody (#ab150387, Abcam) at 1:5000 dilution in 5% non-fat milk powder in 1X TTBS. Each membrane was washed four times in 1X TTBS, incubated with horse radish peroxidase (HRP) conjugated goat anti-rabbit immunoglobulin antibody (1:2000, DAKO, Glostrup, Denmark; #P0448) for 1hr at RT and then washed four times in 1X TTBS. Antigenic bands were visualised using Pierce Supersignal West Pico chemiluminescent substrate (Thermo Scientific, Waltham, MA, USA) and the chemiluminescent intensity measured with a UVP BioSpectrum imaging system (Analytik Jena, Jena, Germany). Sum pixel intensities for mGluR2 were measured using the VisionWorks® Image Acquisition Analysis Software.

To measure β-actin, the nitrocellulose membranes were washed in distilled water for 5 min at RT and then blocked with 1X TTBS for 1hr at RT. The membranes were then incubated with mouse anti-β-actin monoclonal antibody (MAB1501, Merck) at 1:200,000 dilution for 1hr at RT and then washed four times in 1X TTBS. Finally, the membranes were incubated with HRP-conjugated goat anti-mouse immunoglobulin antibody (1:4000, DAKO, Glostrup, Denmark; #P0447), washed four times in 1X TTBS and the antigenic bands visualised and quantified as per the GRM2 protocol.

All data were converted to the sum intensity of GRM2 normalised to the sum intensity of β-actin.

2.3. Statistics

Variance in demographic, clinical and CNS related data with diagnoses was assessed using one-way ANOVA and post hoc Dunnett's tests comparing diagnostic groups to controls whilst frequency of gender and suicide completion was compared using the χ^2 test (GraphPad Prism).

As GRM2 data was normalised β-actin (Dean et al., 2016b), all experimental data were analysed using the Mann-Whitney test in GraphPad Prism or a non-parametric one way ANOVA with appropriate covariates (Dean et al., 2016b; Quade, 1967) using Minitab 18. Spearman's-rank correlations were used to identify correlations between levels of cortical GRM2 and demographic, clinical and CNS processing data.

3. Results

3.1. Antibody validation

The strongest immunogenic band in human cortex for the rabbit anti-GRM2 monoclonal antibody was ~95 kilodaltons (Supplementary Fig. 1), GRM2 predicted molecular weight. The antibody showed no immunogenic reaction with human serum or when human cortex was probed in the absence of either primary or secondary antibody. In addition, the immunogenic band was present in the membrane, but not the cytosolic, fraction of human cortical homogenate; consistent with GRM2 being a membrane bound protein (Niswender and Conn, 2010).

3.2. Potential study confounds

Age ($p = 0.98$), gender ratio ($p = 0.92$), CNS pH ($p = 0.94$), PMI ($p = 0.66$) or DI ($p = 0.60$) did not vary with diagnoses (Table 1). Suicide completion varied with diagnoses, whether or not data from all subjects or just those with psychiatric disorders were analysed.

Levels of GRM2 were higher in BA 46, but not BA 24, from males compared to females (Fig. 1A, Supplementary Table 1A) and suicide completers compared to non-suicide (Fig. 1B, Supplementary Table 1B). There was no inter-relationship between the levels of GRM2 in males and in suicide completers (Supplementary Table 1D and E). There were no significant correlations between levels of GRM2 and age (BA 24 $r = -0.06$, $p = 0.66$; BA 46 $r = -0.16$, $p = 0.23$), CNS pH (BA 24

Table 1
Relevant details relating to the demographics and CNS collection details for subjects with schizophrenia, major depressive disorders, bipolar disorders, schizophrenia and age / gender matched controls used in this study.

	Gender (f / m)	Age (yr)	CNS pH	PMI (Hr)	Suicide (Y / N)	Duration of illness (yr)
Controls	8 / 7	61 ± 3.3	6.28 ± 0.06	44 ± 4.2	0 / 15	
Bipolar disorders	7 / 8	58 ± 3.2	6.29 ± 0.04	39 ± 3.8	5 / 10	18 ± 3.1
Major depressive disorders	8 / 7	60 ± 4.0	6.56 ± 0.04	44 ± 3.9	12 / 3	17 ± 2.4
Schizophrenia	8 / 7	56 ± 3.9	6.27 ± 0.06	47 ± 51	6 / 9	21 ± 3.4
F or χ^2	0.2	0.16	0.13	0.54	20.5	
d.f.	3	3,64	3,64	3,64	3	
p	0.98	0.92	0.94	0.66	0.001	
Psychiatric cases only F of χ^2					7.65	0.51
d.f.					2	2,47
p					0.02	0.60

Abbreviations: f = female, gms = grams, Hr = hours, m = male, N = no, Y = yes, yr = years.