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Density of metabotropic glutamate receptors 2 and 3 (mGluR2/3) in the dorsolateral prefrontal cortex does not differ with schizophrenia diagnosis but decreases with age

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ABSTRACT

Metabotropic glutamate receptors 2 and 3 (mGluR2/3) have been shown as efficient targets for antipsychotic intervention. We therefore investigated the receptor density of mGluR2/3 in the dorsolateral prefrontal cortex (dlPFC; Brodman area 46) of schizophrenia/schizoaffective patients (n = 37) and matched controls (n = 37) using receptor autoradiography. No difference in mGluR2/3 density was identified in relation to schizophrenia diagnosis. Overall and in individual groups, a negative correlation of mGluR2/3 density and age at death has been found. These and previous results suggest that density of mGluR2/3 in the dlPFC is less likely to impact on the efficiency of the mGluR2/3 agonist in treating schizophrenia symptoms.

1. Introduction

Schizophrenia is a highly complex brain disorder and its underlying mechanisms are still not understood. With the strong implication of glutamatergic neurotransmission, metabotropic glutamate receptors 2 and 3 (mGluR2/3) have been examined as potential new targets for antipsychotic treatment (Harrison et al. 2008; Krivoy et al. 2008; Swanson et al., 2005). In both animal and human studies, the highly selective agonist for mGluR2/3, LY404039, was shown to have antipsychotic potential (Patil et al., 2007; Rorick-Kehn et al. 2007b). Intriguingly, no appreciable affinities for other glutamate receptor subtypes and transporters neither for non-glutamate receptors, such as dopamine or serotonin, were found for this compound (Rorick-Kehn et al., 2007a). The efficiency and specificity of the mGluR2/3 agonist make alterations in the density of mGluR2/3 in schizophrenia-relevant brain regions highly likely.

Cognitive impairments in schizophrenia have been associated with a dysfunction of the dorsolateral prefrontal cortex (dlPFC) (Callicott et al., 2000; Eisenberg and Berman 2010); increased prefrontal glutamate concentrations have been found in a subgroup of schizophrenia patients (Olbrich et al., 2008). Using post-mortem brain tissue of schizophrenia patients an earlier study did not find changes in the protein expression of mGluR2/3 in the dlPFC (Crook et al., 2002). A recent study showed, however, a higher expression of mGluR2 than mGluR3 in the dlPFC and a lower expression of mGluR3 in the dlPFC of patients (Ghose et al., 2009); receptor binding density, however, has not been studied.

We therefore studied mGluR2/3 density in post-mortem brain tissue of the patient cohort of the Schizophrenia Research Institute (SRI) comprising 37 patients with schizophrenia diagnosis (including 7 schizoaffective patients) and 37 matched controls to identify the potential role of dlPFC mGluR2/3 in schizophrenia.

2. Methods

2.1. Post-mortem brain tissue

For the present study, post-mortem brain tissue of the dlPFC (Brodman Area 46) of 37 schizophrenia/schizoaffective patients and 37 matched controls was used. All research was approved and conducted under the guidelines of the Human Research Ethics Committee at the University of Wollongong (HE99.222) and University of New South Wales (HREC 07261). Clinical assessments, selection of cases and matched controls, assessment of tissue quality and preparation of slide-mounted coronal tissue sections (14 μm) were performed by the Tissue Resource Centre (TRC) and the Schizophrenia Research Institute (SRI) (Weickert et al., 2010). The

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cohort had, amongst others, the following demographic measures (means ± SEM; for more details see (Weickert et al., 2010)):

Age at death (years): controls 51.1 ± 2.40, schizophrenia cases 51.3 ± 2.32;
Age at death range: controls 18–78 years, schizophrenia cases 27–75 years;
pH (dlPFC): controls 6.66 ± 0.29, schizophrenia cases 6.61 ± 0.30;
Post-mortem interval (hours): controls 24.8 ± 10.97, schizophrenia cases 28.8 ± 14.07;
RNA integrity number: controls 7.3 ± 0.57, schizophrenia cases 7.3 ± 0.58;
Gender distribution: controls 30 men and 7 women, schizophrenia cases 24 men and 13 women;
Hemispheres used: controls 23 right and 14 left, schizophrenia cases 17 right and 20 left;
Mean daily intake of antipsychotics as chlorpromazine equivalent (mg): controls 0 ± 0, schizophrenia cases 702 ± 90;

All experiments and analysis were done blind to the clinical details of each case.

2.2. Receptor Autoradiography for mGluR2/3 density

Receptor autoradiography on mGluR2/3 was performed using [^{3}H]LY354740 based on the protocol described previously (Richards et al., 2005). Two slides per case were pre-incubated for 2 × 10 min in a buffer solution (50 mM Tris buffer, 2 mM $MgCl_2$, 2 mM $CaCl_2$; pH = 7) at room temperature (RT). Thereafter, the slides were incubated for 60 min at RT in buffer solution containing 50nM [^{3}H]LY354740. As controls, adjacent brain sections of 6 selected cases (3 patients vs. 3 matched controls) were incubated with 50nM [^{3}H]LY354740 in the presence of 0.01 mM DCG-IV to determine non-specific binding. Following incubation the slides were washed in cold (4 °C) washing buffer (50 mM Tris buffer, pH7) for 2 × 30 s and 1 × 1 min.

2.3. Receptor density quantification

Quantification was performed using the Beta-Imager and B vision+ program (BioSpace, France). Cortical regions were analysed according to histological standards (provided by Weickert et al., 2010 see Fig. 1).

Four schizophrenia cases (3 schizophrenia, 1 schizoaffective diagnosis) and 1 control case were excluded from analysis as binding data deviated more than 30% from the overall standard deviation.

2.4. Statistical analysis

All data was analysed using SPSS (17.0). Data was tested for normality (Lilliefors test) and homogeneity (Levene's test). Student's *t*-test was used to compare the levels of radioligand binding for schizophrenia/schizoaffective patients vs controls, schizophrenia patients vs controls, schizoaffective patients vs controls, schizoaffective vs schizophrenia patients as well as gender differences and manner of death. Bonferroni correction was used to correct for multiple testing in case of significance. Spearman's correlation was used to test for any effects of continuous descriptive variables including age at death, pH (prefrontal cortex), post mortem interval, RNA integrity (RIN), freezer storage time, brain weight, brain volume, chlorpromazine equivalent (mg) of daily average, lowest, highest, lifetime and last recorded

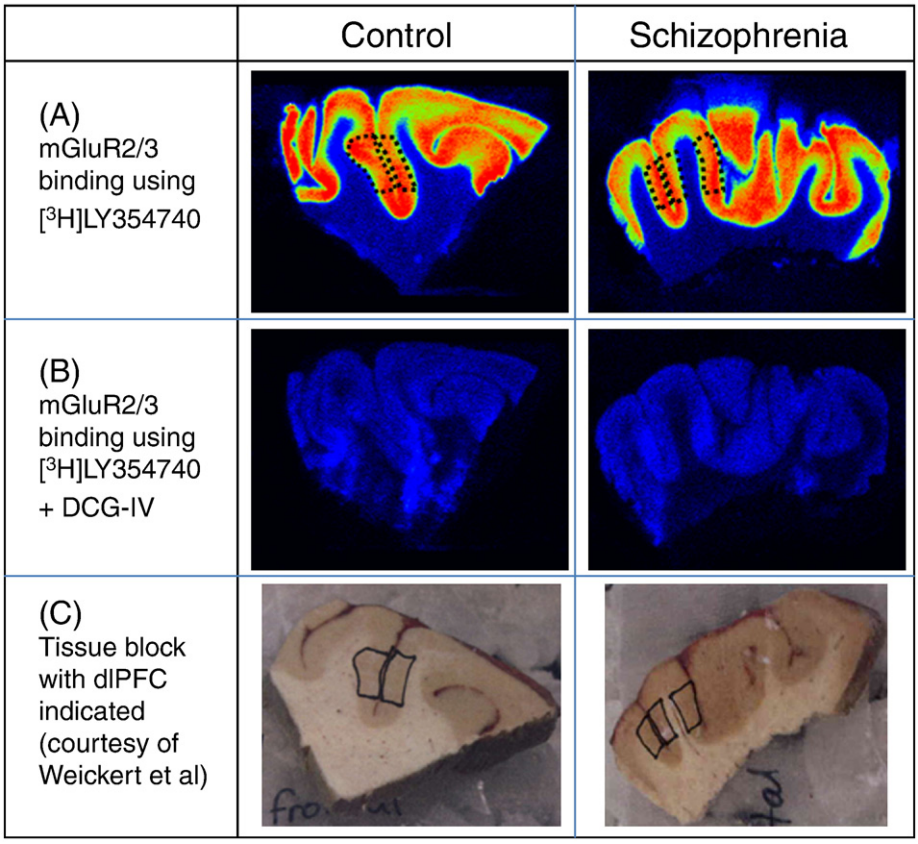

Fig. 1. (A) Receptor autoradiographs using [3 H]LY354740 to label metabotropic glutamate receptors 2 and 3 (mGluR2/3) in the dorsolateral prefrontal cortex (dlPFC) of control subjects and schizophrenia/schizoaffective patients, obtained by beta imager scanning. No difference in mGluR2/3 density in the dlPFC was found due to diagnosis. (B) Addition of the selective mGlu2/3 agonist DCG-IV prevented radioactive labelling of mGluR2/3 evidencing the specificity of [3 H]LY354740 binding to mGluR2/3. (C) Analysis was based on histological identification of the dlPFC provided by Weickert et al. (for reference see Weickert et al. 2010).