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Research report

Lower [3H]LY341495 binding to mGlu2/3 receptors in the anterior cingulate of subjects with major depressive disorder but not bipolar disorder or schizophrenia

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ABSTRACT

Introduction: The glutamatergic system has recently been implicated in the pathogenesis and treatment of major depressive disorders(MDD) and mGlu2/3 receptors play an important role in regulating glutamatergic tone. We therefore measured cortical levels of mGlu2/3 to determine if they were changed in MDD.
Methods: Binding parameters for [^{3}H]LY341495 (mGlu2/3 antagonist) were determined to allow optimized *in situ* binding with autoradiography to be completed using a number of CNS regions. Subsequently, density of [^{3}H]LY341495 binding was measured in BA24(anterior cingulate cortex), BA17(visual cortex) and BA46(dorsolateral prefrontal cortex) from subjects with MDD, Bipolar Disorder(BPD), Schizophrenia(SCZ), and controls, as well as rats treated with imipramine (20 mg/kg), fluoxetine (10 mg/kg), or vehicle.
Results: mGlu2/3 are widely expressed throughout the brain with high levels observed in cortex. [^{3}H]LY341495 binding was significantly lower in BA24 from subjects with MDD (mean ± SEM = 141.3 ± 14.65 fmol/ETE) relative to controls (184.9 ± 7.76 fmol/ETE; Cohen's $d = 1.005$, $p < 0.05$). There were no other differences with diagnoses, and chronic antidepressant treatment in rats had minimal effect on binding.
Limitations: Using this approach we are unable to determine whether the change represents fluctuations in mGlu2, mGlu3, or both. Moreover, using postmortem tissue we are unable to dissociate the irrevocable confound of suicidality upon binding levels.
Conclusion: We have demonstrated lower [^{3}H]LY341495 binding levels in MDD in BA24-a brain region implicated in depression. Moreover we show that the lower levels are unlikely to be the result of antidepressant treatment. These data suggest that levels of either mGlu2 and/or mGlu3 are affected in the aetiology of MDD.

1. Introduction

The glutamatergic system is essential to normal brain function. Glutamate receptors can be ionotropic ligand gated channels or metabotropic G protein coupled receptors (mGlu), the latter of which comprises 8 receptors that have been classed into 3 subgroups based upon their structure and function (Conn and Pin, 1997). Class I couple to Gq, activating phosphoinositide hydrolysis and second messenger signaling via PLC. Class II couple to Gi/o, negatively regulating activity of adenyl cyclase, as do Class III receptors (for review see (Ferraguti and Shigemoto, 2006)). Glutamate receptors have broad and overlapping expression patterns within the brain and, in this way, exert influential control over neural and neuronal activity (Neki et al., 1996a, 1996b; Ohishi et al., 1998) reviewed in (Ferraguti and Shigemoto, 2006).

Dysregulation of the glutamatergic system has been widely implicated in psychiatric disease (Ouellet-Plamondon and Tony, 2012). The most clinically relevant and reproduced examples of the impact of glutamatergic disruption are the psychotomimetic "schizophrenia-like" symptoms produced by ketamine, an antagonist at ionotropic N-methyl-D-Aspartate (NMDA) receptors (Krystal et al., 1994). Extending these findings, ligands for Class II

Abbreviations: mGlu:, metabotropic glutamate receptor; BPD:, Bipolar Disorder; MDD:, Major Depressive Disorders; SCZ:, Schizophrenia; CTL:, Control; PFC:, Prefrontal Cortex; NMDA:, N-methyl-D-Aspartate; DIBS:, Diagnostic Instrument for Brain Studies; BA:, Brodmann's Area; PMI:, Postmortem Interval; r/t:, room temperature; TB:, total binding; NSB:, non specific binding; ETE:, estimated tissue equivalent; IMI:, imipramine; FLX:, fluoxetine

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metabotropic glutamate receptors (Class II mGlu), comprising mGlu2 and mGlu3, have been shown to mitigate the psychotropic effects of NMDA antagonism (Homayoun et al., 2005; Moghaddam and Adams, 1998; Pehrson and Moghaddam, 2010; Verma and Moghaddam, 1998). Interestingly, ketamine also possesses antidepressant activity (Berman et al., 2000; Trullas and Skolnick, 1990), and mGlu2/3 (and second messenger signaling via mTOR and GSK) appear to be required for this response (Dwyer et al., 2012; Li et al., 2010).

Significantly, there have been a number of studies on the NMDA receptor, the site of ketamine's action, in postmortem CNS from people with schizophrenia and depression (for review see (Coyle and Tsai, 2004;Licznerski and Duman, 2013)). Focusing on mood disorders, lower levels of NMDA receptor subunits (NR2A and NR2B) have been reported in the prefrontal cortex of subjects with major depressive disorder (MDD) (Beneyto et al., 2007), while higher NR2A levels have been reported in the amygdala (Karolewicz et al., 2009), demonstrating regional specificity for these changes. Interestingly, increases in NR2D have been reported in the frontal pole (Dean et al., 2015) suggesting a shift in NMDA receptor subtype, rather than an overall change in NMDA receptor levels. This aligns with studies that report no changes in NMDA receptor binding in major depressive disorders (Holemans et al., 1993).

By contrast, only a few studies have addressed the status of class II mGlu in the CNS of people with mood disorders, with mixed findings. Higher mGlu2/3 expression has been reported in the prefrontal cortex in MDD (Feyissa et al., 2010), using western blotting, while autoradiographic binding experiments report no changes in absolute levels of [^{3}H]LY341495 to mGlu2/3 in BA24 (Matosin et al., 2014). Importantly, a systematic study of [^{3}H]LY341495 binding in human brain has not been performed, complicating the contextualization of these findings.

Together these findings highlight the complexity of the disorder and the need for further clarification of the state of components of the glutamatergic system, and a better understanding of how the tenuous balance of glutamatergic function is altered in psychiatric disorders.

In order to add to these limited data and provide further insight into the role of mGlu2/3 in psychiatric disorders, we first established the binding parameters for [^{3}H]LY341495 to human CNS and performed a descriptive assessment of binding to mGlu2/3 across human brain regions. We then measured levels of [^{3}H]LY341495 binding in the anterior cingulate cortex, visual cortex, and dorsolateral prefrontal cortex of subjects with major depressive disorders, bipolar disorders, schizophrenia, and age and sex matched controls. Finally, we measured [^{3}H]LY341495 binding in the CNS of rats after treatment with fluoxetine or imipramine to determine whether modulation of these receptors is a mechanism of action of these drugs.

2. Methods

2.1. Tissue collection: human

Following consent from the Ethics Committee of the Victorian Institute of Forensic Medicine and the Mental Health Research and Ethics Committee of Melbourne Health, human postmortem brain samples were obtained from the Victorian Brain Bank Network (VBBN). Left hemispheres of human CNS were collected postmortem, rapidly sliced, and frozen to 80 °C as described previously (Dean et al., 1999). For all cases, psychiatric diagnoses were performed according to DSM IV criteria following a review of clinical records using the Diagnostic Instrument for Brain Studies (DIBS), a structured instrument allowing consensus psychiatric diagnoses to be made after death (Hill et al., 1996; Roberts et al., 1998). For all non-psychiatric cases, an extensive review of case histories along with enquiries with treating clinicians and families was undertaken to exclude any history of psychiatric illness. The CNS from all potential cases was subjected to a neuropathological examination, any obvious pathology was a criterion for exclusion. During the case history review, suicide completion was recorded when suicide was listed as contributing to death in the Coroner's report. Duration of illness (DI) was calculated as time from first clinical presentation to a psychiatric service, to death. All cadavers were stored at 4 °C within five hours of death. During case history review age at death, gender, post mortem interval (PMI), and brain pH (Kingsbury et al., 1995) were determined (see Table 1). With regards to PMI, where death was witnessed, PMI was from time of death to autopsy but if the death was not witnessed, time of death was taken as the mid-point between the time the donor was last observed alive (maximum of 5 h was allowed), and the time found dead. Cohorts were matched as closely as possible for age, PMI, and brain pH (Table 1).

Table 1
Cohort demographics.

	CTL ($N=15$)	BPD ($N=15$)	MDD ($N=15$)	SCZ ($N=15$)
Age	60.13 (3.40)	59.20(3.14)	59.30(4.20)	55.90(4.05)
Male:Female	7:8	7:8	7:8	7:8
PMI	43.54 (4.31)	37.55(3.99)	44.06(4.02)	45.11(3.89)
pH	6.28(0.06)	6.26(0.06)	6.56(0.04)*	6.26(0.06)
Suicides	0	4	13	6

Demographics for the human postmortem cohorts ($N=15$ per diagnostic group) are described above, with continuous variables presented as **mean (standard error of the mean)**. No differences were observed across diagnoses for Age, Sex, or post mortem interval. A significant difference in pH was identified, with the MDD cohort demonstrating slightly higher pH than the other groups. Suicide, an irreconcilable confound, was also unsurprisingly found to vary across groups. Abbreviations: Control (CTL), Bipolar Disorder (BPD), Major Depressive Disorder (MDD) and Schizophrenia (SCZ), Postmortem Interval (PMI).

For the descriptive study investigating levels of [^{3}H]LY341495 binding across brain regions, BA9 (bridging the dorsolateral prefrontal cortex and the medial prefrontal cortex) was bounded by the lateral surface of the frontal lobe, comprising the middle frontal gyrus superior to the inferior frontal sulcus. BA10, the anterior-most subregion of the frontal cortex, was defined as the most rostral portion of the superior frontal gyrus. The ventral boundary was defined by the superior rostral sulcus. Caudate putamen and substantia nigra are distinct regions that were analysed whole. The hippocampus, also a discrete region, was taken at the level of the lateral geniculate body. 3–5 subjects were used for each brain region.

For the study of psychiatric disorders, dorsolateral prefrontal cortical (Brodmann's area (BA) 46: the lateral surface of the frontal lobe approximately constrained to the middle third of the middle frontal gyrus and the most rostral inferior frontal gyrus), anterior cingulate cortical (BA 24: the ventral anterior cingulate gyrus, around the most anterior aspect of the corpus callosum) and visual cortex (BA 17: the region in the occipital cortex in which the band of Gennari is evident) was obtained from cohorts of 15 people who had either a major depressive disorder (MDD), bipolar disorder (BPD), schizophrenia (SCZ), or subjects with no history of psychiatric illness (controls; CTL). The cingulate cortex was chosen for its known role in MDD (Belzung et al., 2014; Hamani et al., 2011), the dorsolateral prefrontal cortex has been widely implicated in MDD, SCZ, and BPD (Bunney and Bunney, 2000; Selemon and Rajkowska, 2003; Koenigs and Grafman, 2009). A literature search was performed to identify a region not impacted in these disorders. While we were unable to identify a completely unaffected cortical region, there was relatively less evidence for BA17 of the