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Metabotropic glutamate receptor 5 binding and protein expression in schizophrenia and following antipsychotic drug treatment

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Abstract

Metabotropic glutamate receptor 5 (mGluR5) has been identified as a potential therapeutic target for schizophrenia, primarily due to its ability to indirectly modulate glutamatergic signalling through the NMDA receptor (NMDAR). Despite its potential, molecular studies characterising mGluR5 in schizophrenia are limited. We therefore aimed to determine if the mGluR5 binding site or protein levels were altered in schizophrenia or by current antipsychotics. Using in-situ radioligand binding and immunoblot, we measured [3H]MPEP binding to mGluR5 and mGluR5 protein density in the post-mortem dorsolateral prefrontal cortex (DLPFC; BA46) of 37 schizophrenia and 37 matched control subjects. Subsequently, we measured [3H]MPEP binding in rat brains following typical (haloperidol) or atypical (olanzapine) antipsychotic treatment (n = 6/group). Subjects with schizophrenia showed no significant alteration in mGluR5 binding density or mGluR5 protein levels. Furthermore, mGluR5 binding in the rat cortex, thalamus, hippocampus and striatum was unaltered by short-, medium- and long-term antipsychotic treatment. Our data suggests that there are no alterations in mGluR5 in schizophrenia subjects. The lack of alteration in mGluR5 binding and protein in schizophrenia is advantageous because its ability to modulate the NMDAR is potentially unhindered, thereby supporting the development of novel antipsychotic agents that work through the mGluR5/NMDAR complex.

Keywords

drug, antipsychotic, following, schizophrenia, expression, 5, metabotropic, binding, treatment, protein, glutamate, receptor, CMMB

Disciplines

Medicine and Health Sciences | Social and Behavioral Sciences

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Title: Metabotropic Glutamate Receptor 5 Binding and Protein Expression in Schizophrenia and following Antipsychotic Drug Treatment

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Abstract

Metabotropic glutamate receptor 5 (mGluR5) has been identified as a potential therapeutic target for schizophrenia, primarily due to its ability to indirectly modulate glutamatergic signalling through the NMDA receptor (NMDAR). Despite its potential, molecular studies characterising mGluR5 in schizophrenia are limited. We therefore aimed to determine if the mGluR5 binding site or protein levels were altered in schizophrenia or by current antipsychotics. Using in-situ radioligand binding and immunoblot, we measured [³H]MPEP binding to mGluR5 and mGluR5 protein density in post-mortem dorsolateral prefrontal cortex (DLPFC; BA46) of 37 schizophrenia subjects (including 7 schizoaffective) and 37 matched controls. Subsequently, we measured [³H]MPEP binding in rat brains following typical (haloperidol) or atypical (olanzapine) antipsychotic treatment (n=6/group). Subjects with schizophrenia (n=30) showed no significant alteration in mGluR5 binding density or mGluR5 protein levels. However subjects with schizoaffective disorder (n=7) displayed a trend towards reduced mGluR5 binding (20%) compared to schizophrenia subjects (p=0.079), suggesting different underlying biochemistry of the disorder. In schizoaffective subjects of depressive subtype (n=4), mGluR5 binding was reduced by 39% compared to controls (p=0.022). Furthermore, mGluR5 binding in the rat cortex, thalamus, hippocampus and striatum was unaltered by short-, medium- and long-term antipsychotic treatment. Our data suggests that there are diagnostic specific alterations in mGluR5, with a molecular distinction between schizophrenia and schizoaffective disorder. The lack of alteration in mGluR5 binding and protein in schizophrenia is advantageous because its ability to modulate the NMDAR is potentially unhindered, thereby supporting the development of novel antipsychotic agents that work through the mGluR5/NMDAR complex.

Keywords

Antipsychotic

Dorsolateral prefrontal cortex

mGluR5

Post-mortem human brain tissue

Schizophrenia

Abbreviations

ADD: antidepressant drug; APD: antipsychotic drug; DLPFC: Dorsolateral prefrontal cortex; mGluR5: metabotropic glutamate receptor 5; MPEP: 6-methyl-2-(phenylethynyl)pyridine; NMDAR: NMDA receptor; SZ: schizophrenia; SZA: schizoaffective; PAM: positive allosteric modulator; PFC: prefrontal cortex; PMI: post-mortem interval; RIN: RNA integrity number.

1. Introduction

Whilst the precise cause of schizophrenia is unknown, evidence suggests dysfunction of glutamatergic signalling contributes to all aspects of schizophrenia symptomatology including negative and cognitive deficits. Although the major theories relate to a hypofunction of the NMDA receptor (NMDAR) (Marek et al., 2010), metabotropic glutamate receptors (mGluR) have also been implicated (Niswender and Conn, 2010). Metabotropic glutamate receptor 5 (mGluR5) is one of eight known glutamate-specific G-protein coupled receptors which has been shown to uniquely modulate NMDAR activity through a physical and functional link (Tu et al., 1999; Alagarsamy et al., 2002; Luccini et al., 2007). Consequently, mGluR5 has become of interest as a potential target for antipsychotic treatment. However, evidence indicates that mGluR5 itself may also play a role in the pathophysiology of schizophrenia (Krivoy et al., 2008).

mGluR5 knock-out mice exhibit deficits in prepulse inhibition, memory deficits, and hyperlocomotion (Kinney et al., 2003; Brody et al., 2004). Similarly, mGluR5 negative allosteric modulators (NAMs) induce social interaction deficits, impaired working memory and reduced instrumental learning (Homayoun et al., 2004; Koros et al., 2006), implying a direct relationship between reduced mGluR5 function and the manifestation of schizophrenia-like symptoms. Moreover, mGluR5 positive allosteric modulators (PAM) are effective in the attenuation of cognitive as well as negative and positive schizophrenia-like symptoms in NMDAR antagonist and dopaminergic animal models. (Vales et al., 2010). These studies highlight a possible role for mGluR5 in schizophrenia pathology as well as the therapeutic potential of targeting mGluR5 to regulate glutamatergic NMDAR signalling, particularly for the treatment of negative and cognitive aspects of schizophrenia symptomatology.

Although animal studies suggest that mGluR5 is involved in the pathology of schizophrenia, studies characterizing mGluR5 in post-mortem human brain tissue have found minimal changes in mRNA (Ohnuma et al., 1998, 2000; Richardson-Burns et al., 2000; Volk et al., 2010) and protein (Gupta et al., 2005; Corti et al., 2011). This suggests that mGluR5 is unaltered in the pathological state, which is beneficial for novel therapeutics that modulate NMDAR signalling through mGluR5. However, alteration of the specific binding site(s) could render these therapeutics unsuitable. Despite this, examination of binding to mGluR5, specifically at the 6-methyl-2-(phenylethynyl)pyridine (MPEP) allosteric binding site (the specific target of mGluR5 modulators), has never before been investigated in schizophrenia.

It is crucial to characterize mGluR5, both in response to the pathological state and to current antipsychotics to decipher not only if it is a viable target for future therapeutics, but also to determine if mGluR5 is already affected by current antipsychotic drugs (APDs). For the first time, we measured the binding density as well as the protein density of mGluR5 in the dorsolateral prefrontal cortex (DLPFC), a brain region specifically involved in cognitive function (Eisenberg and Berman, 2010), in a large cohort of schizophrenia and control subjects. Furthermore, we analysed mGluR5 binding following typical and atypical APD treatment to determine if these agents have short- or long-term adaptive effects on mGluR5.

2. Methods

2.1. Human Post-Mortem Brain Samples

Tissue was acquired from the New South Wales Tissue Resource Centre (TRC) and its use in this investigation was approved by, and conducted under the guidelines of the Human Research Ethics Committees at the University of Wollongong (HE99/222) and the University of New South Wales (HREC07261). 37 schizophrenia subjects (including 7 schizoaffective) diagnosed according to DSM-IV and 37 controls were matched by the TRC and the Schizophrenia Research Laboratory (SRL) (Table 1). APD treatment premortem was standardised to lifetime chlorpromazine equivalent for each patient. Antidepressant drug (ADD) treatment history was also specified on a non-numeric scale (i.e. yes/no). Clinical and demographic characterisation of the cohort has been detailed previously (Weickert et al., 2010).

2.2. Human Tissue Dissection and Preparation

Anatomical identification of the DLPFC and preparation of the tissue has been previously described in detail (Fung et al., 2010; Weickert et al., 2010). For receptor autoradiography experiments, the DLPFC was coronally sectioned at -18°C, at 14µm thickness, thaw-mounted onto gelatinized slides and stored at -80°C. For western blot experiments, 40mg of frozen DLPFC tissue was homogenised in 400µL of buffer, containing 50mM Tris pH 7.5, 50% glycerol and 1:20 protease inhibitor cocktail.

2.3. Receptor Autoradiography

mGluR5 binding was based on the protocol previously described by Samadi et al. (2008). Two sections per case were preincubated for 15min at room temperature (pH7.5) in 50mM TrisNaCl. Slides were then incubated in a solution containing 10nM [³H]MPEP (specific mGluR5 antagonist; specific activity 60Ci/mmol; American Radiolabelled Chemicals, USA) and 50mM TrisNaCl for 90min at room temperature. Adjacent sections were incubated in the same solution with the addition of 10µM unlabelled MPEP (Sigma, Australia) to determine non-specific binding. Slides were washed in TrisNaCl (4°C, pH 7.5) for 2x 3min and briefly dipped in 4°C MilliQ-water.

Sections were exposed to tritium sensitive Kodak Biomax MR film (Kodak, UK) for 8 weeks, together with [³H] microscale autoradiographic standards (Amersham). Films were developed using the AGFA CP1000 film developer (Agfa-Gevaert N.V., Mortsel, Belgium), scanned using a GS800 densitometer (BioRad, USA) and analysed using Multi-Analyst software (BioRad). Histological standards for quantitative analysis were previously defined by immunostaining (Yang et al., 2010). Experiments and quantification were performed blind to diagnosis.

2.4. Western Blot

Protein density was measured within homogenates using mGluR5 polyclonal antibody (1:250, ABCAM ab27190). Samples were run in duplicate at a concentration of 5µg of total protein. Densitometry values for each of the samples were normalized to the respective pooled sample and beta-actin density, which has been reported as a reliable standard in this cohort (Weickert et al., 2010). This protocol is extensive and has been included in supplementary materials (S1).

2.5. Animal Housing, Treatment and Receptor Autoradiography Analysis of Rat Tissue

All animal experiments in this study were approved by the University of Wollongong Animal Ethics Committee (AE10/18) and complied with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. 54 adult (10-weeks of age) male Sprague-Dawley rats were purchased from the Animal Resource Centre (Perth, Australia) and housed under standard conditions with food and water available ad libitum. After one week of acclimatization, rats (n=6/group) were fed prepared food pellets containing either:

- (1) Typical antipsychotic, haloperidol (0.1mg/kg, 3 times/day),
- (2) Atypical antipsychotic, olanzapine (1mg/kg, 3 times/day),
- (3) Vehicle (control),

for 8, 16, or 36 days. APD doses were chosen to model a clinical setting (S2).

Rats were euthanized 48 hours following the final treatment using carbon dioxide asphyxiation. Brains were rapidly removed and sectioned at -17°C using a cryostat (Leica CM1950, Germany) into 14µm coronal sections at the levels of the PFC (Bregma+3.72mm), caudate-putamen (striatum; Bregma+1.2mm), hippocampus and thalamus (Bregma-3.14mm). Sections were thaw-mounted onto Polysine™ microscope slides (Menzel GmbH & Co KG, Germany) and stored at -20°C.

[³H]MPEP binding to mGluR5 was performed in triplicate according to the protocol described above (section 2.3). Identification of regions for quantification was based on a standard rat brain atlas (Paxinos and Watson, 2007). Experiments and quantification were performed blind to treatment group.

2.6. Statistical Analysis

Parametric tests were employed to analyse the differences between patients with schizophrenia and control subjects as data was normally distributed. Independent t-tests and ANOVA (followed by Tukey post-hoc) were used to compare differences between mGluR5 binding/protein within the diagnostic groups. Correlations with demographic and post-mortem data were scrutinized, and these factors (pH/age at death/PMI/freezer storage time/RIN/brain weight/age of disease onset/duration of illness/lifetime chlorpromazine equivalent) were analysed for their effects over the data through ANCOVA testing. T-tests were used to determine the effects of antidepressant history/toxicology on binding/protein within the schizophrenia subjects. In the animal study, two-way MANOVA was performed for data within each brain region (PFC/caudate/putamen/thalamus/hippocampus) considering treatment type and duration. Statistical analyses were performed with SPSS software (19.0).

3. Results

3.1 Analysis of Demographic and Clinical Data

One case (control) was excluded from analysis for [³H]MPEP binding due to technical issues. Despite this, mean pH, age at death, PMI, freezer storage time, brain weight and RNA integrity number (RIN) did not differ between schizophrenia (including schizoaffective) and control groups ($-1.525 < t < 1.264$, $0.132 < p > 0.955$) or schizophrenia, schizoaffective and control groups ($0.003 < F < 1.957$, $0.149 < p > 0.997$). In addition, age of disease onset, duration of illness, and lifetime exposure to APD (lifetime chlorpromazine) did not differ between schizophrenia and schizoaffective subjects ($-1.408 < t < 0.552$, $0.168 < p > 0.762$). All data were normally distributed.

3.2. Diagnosis Related Effects

[³H]MPEP binding density was highly expressed, with a homogenous distribution across the cortical layers (Fig. 1a). Independent t-tests revealed no significant change in [³H]MPEP binding density in all schizophrenia subjects (including schizoaffective subjects) compared to controls ($t_{71} = -0.003$, $p = 0.998$). ANCOVA confirmed this finding ($F_{1,65} = 0.708$, $p = 0.403$). A trend was observed whereby binding density was reduced by 20% in schizoaffective (SZA) ($n = 7$) compared to schizophrenia subjects ($n = 29$) (SZ: 10.07 ± 0.48 , SZA: 8.01 ± 1.19 ; $t_{34} = 1.807$, $p = 0.079$). On further examination, [³H]MPEP binding density showed a significant 39% reduction in schizoaffective subjects of depressive subtype ($n = 4$) compared to controls ($n = 37$) ($p = 0.023$) and a 42% reduction compared to schizophrenia subjects ($p = 0.011$) (Fig. 2a). Despite the clear reduction observed in Fig. 2a, this analysis is preliminary due to sample size constraints. No differences in [³H]MPEP binding were observed in other schizophrenia diagnostic subgroups (residual/disorganized/undifferentiated/paranoid) compared to controls ($F_{4,61} = 0.897$, $p = 0.471$).

mGluR5 protein was detected in the DLPFC as a single band at the expected molecular weight and was normalized to beta-actin (Fig. 1b). Independent samples t-tests showed no significant changes in mGluR5 protein between schizophrenia subjects and controls (CT: 0.91 ± 0.4 , SZ: 0.91 ± 0.03 ; $t_{72} = 0.081$, $p = 0.935$), as confirmed by ANCOVA ($F_{1,66} = 0.244$, $P = 0.784$). Unlike the [³H]MPEP binding data, there was no significant change in mGluR5 protein between schizoaffective subtypes, schizophrenia subjects and controls ($F_{3,70} = 0.261$, $p = 0.771$) (Fig. 2b). No significant changes in receptor protein levels were observed between any of the schizophrenia diagnostic subgroups compared to controls ($F_{4,62} = 0.165$, $P = 0.955$). Although it appeared that there was an outlier within the schizoaffective subjects of depressive type (see Fig. 2b), the data point was not a statistical outlier. Additionally, removal of the data point did not affect the significance of the data (data not shown).

3.1.3. Effects of Demographic and Clinical Variables

Examination revealed no main effect of hemisphere on mGluR5 binding ($F_{1,71} = 0.105$, $p = 0.747$) or protein ($F_{1,72} = 2.010$, $p = 0.161$) and no interaction between hemisphere and diagnosis ([³H]MPEP: $F_{3,69} = 0.345$, $p = 0.559$; mGluR5 protein levels: $F_{3,70} = 0.732$, $p = 0.537$). In addition, no effect of gender was found on [³H]MPEP binding ($F_{1,71} = 0.009$, $p = 0.924$) or mGluR5 protein ($F_{1,72} = 0.886$, $p = 0.350$) and no interaction between gender and diagnosis on mGluR5 protein ($F_{3,70} = 1.918$, $p = 0.170$). Whilst a significant interaction between gender and diagnosis was found for [³H]MPEP

binding density ($F_{3,69}=5.308$, $p=0.024$), post-hoc tests failed to detect significant differences within the four groups (schizophrenia male/schizophrenia female/control male/control female).

Pearson's correlations for continuous variables (pH/age at death/PMI/freezer storage time/brain weight/RIN/age of disease onset/duration of illness/lifetime chlorpromazine equivalent) are presented in Table 2. mGluR5 binding correlated positively with age at death in control subjects, an effect that was not seen in schizophrenia or schizoaffective subjects. mGluR5 binding correlated negatively with pH in control and schizoaffective subjects, but not in schizophrenia subjects. mGluR5 binding also correlated negatively with brain weight in schizophrenia subjects, and trending in schizoaffective subjects. mGluR5 protein levels correlated negatively with PMI in control and schizophrenia subjects (and trending in schizoaffective subjects) and positively with freezer storage time in control and schizophrenia subjects. These variables were accounted for in the ANCOVA analyses. Furthermore, as stated above, age at death, RIN, pH, brain weight and PMI did not differ between control, schizophrenia and schizoaffective subjects. Importantly, no correlation was found between mGluR5 binding/protein and lifetime APD dosage, as measured by lifetime chlorpromazine equivalent. There was no effect of ADD treatment history (yes: $n=19$, no: $n=18$; binding: $t_{34}=-1.039$, $p=0.306$; protein: $t_{35}=0.044$, $p=0.965$) or ADD toxicology post-mortem (yes: $n=12$, no: $n=25$; binding: $t_{34}=-0.531$, $p=0.598$; protein: $t_{35}=1.037$, $p=0.307$).

3.2. [3H]MPEP binding to mGluR5 in APD Treated Rat Brain Tissue

[3H]MPEP binding density was highly expressed in all examined rat brain regions (Fig. 3). Two-way MANOVA comparing haloperidol, olanzapine and control treated rats for different treatment durations (8, 16 or 36 days) revealed no significant main effects of treatment or treatment duration, or interaction between the two factors in the PFC, hippocampus, striatum or thalamus (Table 3).

4. Discussion

mGluR5 is a potential therapeutic target for several neuropsychiatric disorders, including schizophrenia (Vinson and Conn, 2012). For the first time we have examined binding to mGluR5 in schizophrenia and schizoaffective patients and in the brains of rats following APD treatment. We report no change in mGluR5 binding or protein density in schizophrenia subjects compared to controls. Furthermore, we found that olanzapine and haloperidol, APDs commonly used in a clinical setting to treat schizophrenia, do not alter mGluR5 binding density. We propose that the lack of change in mGluR5 binding and protein levels in schizophrenia subjects is beneficial, as the effectiveness of novel mGluR5-targeted therapeutics will not be affected. In addition, confirmation that current schizophrenia therapeutics do not alter mGluR5 further supports that this should be considered as a novel drug target, especially to treat those symptoms that current therapeutics do not.

4.1. mGluR5 binding density and protein levels are unaltered in the DLPFC in Schizophrenia

In line with our results, no change in mGluR5 protein (Gupta et al., 2005; Corti et al., 2011) or mRNA (Ohnuma et al., 1998, 2000; Richardson-Burns et al., 2000; Volk et al., 2010) levels have been reported in the PFC of schizophrenia subjects. Together, this suggests that mGluR5

expression is not altered in the pathology of schizophrenia, at least within the PFC. The DLPFC is a sub-region of the PFC highly involved in cognitive function, and disruptions to circuitry within this brain region are believed to underlie the cognitive deficits seen in patients with schizophrenia (Eisenberg and Berman, 2010). Our findings suggest that there are no pathological alterations of mGluR5 expression in the DLPFC, and therefore mGluR5 expression does not contribute to these cognitive deficits. These findings may extend to other mGluRs, such as mGluR2/3, which have been previously reported as unchanged in the same cohort (Frank et al., 2011).

There is strong evidence that dysfunction of glutamatergic NMDARs underlies many of the cognitive, negative and positive symptoms of schizophrenia (Kantrowitz and Javitt, 2010). Due to the global distribution and rapid synaptic transmission of the NMDAR, it is not suited as a therapeutic target as its effects are widely excitotoxic (Ellenbroek, 2012). However mGluR5 and NMDAR are connected by a scaffolding link (Tu et al., 1999) and are known to functionally interact, particularly in the PFC (Pisani et al., 2001; Alagarsamy et al., 2002, 2005; Benquet et al., 2002; Homayoun and Moghaddam, 2006). Hence, mGluR5 is of current interest as a novel therapeutic target for the treatment of schizophrenia. Studies of mGluR5 positive allosteric modulators (PAMs) have repeatedly reported antipsychotic-like effects in rodent models of schizophrenia (see Vinson and Conn, 2012), which have been shown, in part, to be mediated through the frontal cortex (Homayoun and Moghaddam, 2008). Most interestingly, PAMs of mGluR5 reverse drug-induced negative and cognitive impairments in rodents, which are aspects of schizophrenia symptomatology mostly untreated by current therapeutics in a large percentage of patients. Specifically, mGluR5 PAMs improve performance in sucrose preference, recognition memory, set-shifting, Y-maze, Morris water maze, inhibitory avoidance learning and conditioned aversion tasks whilst also reversing hyperlocomotive and PPI deficits in phencyclidine, apomorphine and/or amphetamine treated rodents, models routinely used to test antipsychotic drug potential (see Vinson and Conn, 2012). Most mGluR5 PAMs interact with the MPEP allosteric binding site (Chen et al., 2007), which was the target of the radioligand used in the present study to characterize mGluR5 binding. As we report that this specific allosteric binding site and mGluR5 protein levels are unaltered in schizophrenia pathology, this suggests that the binding potential of mGluR5 PAMs will not be affected in patients with schizophrenia.

We report a positive correlation between mGluR5 binding and age at death in control subjects but not in schizophrenia or schizoaffective subjects. Whilst this suggests that an age-interaction may be concealing a pathological effect, our findings are in contrast to other studies on mGluR5 binding (Deschwenden et al., 2011), protein (Gupta et al., 2005; Corti et al., 2011) and mRNA (Ohnuma et al., 1998; Richardson-Burns et al., 1999; Ohnuma et al., 2000; Volk et al., 2010), which report that mGluR5 remains stable with age. Furthermore, Corti et al. (2011) reported a negative correlation between mGluR5 protein and age in both control and schizophrenia subjects. As we are the first to examine [³H]MPEP binding in the schizophrenia brain, this association may be genuine; however, considering it is in contrast to previous protein and mRNA studies, these results must be interpreted with caution.

4.2. mGluR5 binding density is reduced in schizoaffective subjects of the depressive type

Although mGluR5 was unaltered in schizophrenia subjects, a significant 39% reduction was observed in [³H]MPEP binding in the DLPFC of schizoaffective subjects of depressive type compared to controls. Importantly, there was no significant effect of ADD treatment history or ADD post-mortem toxicology on mGluR5 binding/protein. Whilst this finding must be interpreted with caution due to the small sample size, it is consistent with a recent study reporting reduced (5.7%) mGluR5 mRNA expression in the PFC of schizoaffective patients (n=14) compared to schizophrenia patients (n=28) (Volk et al., 2010). Furthermore, Deschwanden et al. (2011) recently reported decreased mGluR5 binding in the PFC of subjects with major depression. It is plausible that the mood component may be driving this consistent reduction in mGluR5 seen in schizoaffective subjects but not schizophrenia subjects. These findings support previous studies indicating that schizophrenia and schizoaffective disorder have differing underlying neurobiologies (Volk et al., 2010; Bychkov et al., 2011), which may indicate the need for tailored therapeutics. Previous studies have often combined schizoaffective with schizophrenia subjects to increase power for analysis. These findings highlight the importance of examining schizoaffective disorder as separate to schizophrenia.

4.3. Effects of Antipsychotic Drug Treatment on mGluR5

A concern with the use of post-mortem human brain tissue is the effects of pharmacotherapy pre-mortem (Hynd et al., 2003; Weickert et al., 2010). In the present study, there was no correlation between mGluR5 binding/protein with APD treatment in the schizophrenia human brain. We examined mGluR5 following varying periods in rats treated with haloperidol or olanzapine. Neither olanzapine nor haloperidol altered mGluR5 binding, although there is evidence to suggest that mGluR5 and D₂ receptors form heterodimers in the striatum (Agnati et al., 2010), indicating a possible mechanism for APD-induced mGluR5 alterations. Our findings are consistent with previous studies that have reported mGluR1 (Volk et al., 2010) and 2/3 (Crook et al., 2002; Gupta et al., 2005) are not altered by current antipsychotic therapeutics. Current APDs are widely effective in treating positive symptoms, however they have minimal effects on negative symptoms and very limited therapeutic potential for cognitive symptoms. As glutamatergic signaling in the PFC is a major contributor to the cognitive impairments associated with schizophrenia (Deakin et al., 1989; Perlstein et al., 2001), our findings support that mGluR5 is unlikely to be affected by current APD treatment. As current therapeutics do not influence mGluR5, this provides further impetus to target this receptor for new therapeutics.

4.4. Conclusion

The present study investigated for the first time (1) diagnosis related alterations of mGluR5 in schizophrenia/schizoaffective disorder and (2) the effects of current antipsychotics on mGluR5 binding. It was demonstrated that mGluR5 binding density was unaltered in schizophrenia, but was greatly reduced (39%) in schizoaffective subjects of depressive type. Due to small power in the schizoaffective group, further investigation is needed in a larger cohort to validate these findings. In line with our hypotheses, APD treatment did not affect mGluR5 binding in an animal model, which we posit is due to APDs having minimal direct effects on glutamatergic signaling and subsequently cognitive function. This study supports the potential of targeting mGluR5 to selectively modulate the NMDAR for the treatment of schizophrenia, particularly negative and cognitive symptoms mediated by the PFC. Further studies are now required to characterize the

chronic effects and efficacy of mGluR5 modulators in animal models, and in due course, a clinical setting.

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