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Differential expression of metabotropic glutamate receptor 2 and 3 in schizophrenia: a mechanism for antipsychotic drug action?

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Abstract

Objective—Preclinical and clinical data implicate the group II metabotropic glutamate receptors (mGluR2 and mGluR3) in the pathophysiology of schizophrenia. Moreover, a recent phase II clinical trial has demonstrated the antipsychotic efficacy of a mGluR2/3 agonist. The current study was designed to distinguish the expression of mGluR2 and mGluR3 receptor protein in schizophrenia and to quantify glutamate carboxypeptidase II (GCPII) in order to explore a role for the metabotropic receptors in schizophrenia therapeutics. GCPII is an enzyme that metabolizes N-acetylaspartylglutamate (NAAG), the only known specific endogenous agonist of mGluR3 in the mammalian brain.

Method—The normal expression levels of mGluR2, mGluR3 and GCPII were determined in 10 regions of the human post mortem brain using specific antibodies. Differences in expression levels of each protein were then examined in the dorsolateral prefrontal (DLPFC), temporal (TC) and motor cortex (MC) in 15 matched cases of schizophrenia and normal controls. Chronic antipsychotic treatment in rodents was conducted to examine the potential effect of antipsychotic drugs on expression of the 3 proteins.

Results—We found a significant increase in GCPII protein and a reduction in mGluR3 protein in the DLPFC in schizophrenia with mGluR2 protein levels unchanged. Chronic antipsychotic treatment in rodents did not influence GCPII or mGluR3 levels.

Conclusions—Increased GCPII expression and low mGluR3 expression in the DLPFC suggest that NAAG-mediated signaling is impaired in this brain region in schizophrenia. Further, these data implicate the mGluR3 receptor in the antipsychotic action of mGluR2/3 agonists.

Keywords

prefrontal cortex; glutamate carboxypeptidase II; mGluR; psychosis; human postmortem

Introduction

A role for glutamate in the pathophysiology of schizophrenia is suggested by the psychomimetic properties of phencyclidine (PCP). PCP and ketamine, antagonists of the glutamate N-methyl-D-aspartate (NMDA) receptor, can cause positive, negative and cognitive symptoms reminiscent of schizophrenia in healthy controls and exacerbate

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symptoms in volunteers with schizophrenia (1,2). The G protein coupled metabotropic glutamate receptors, mGluR2 and mGluR3, distributed at the presynaptic, postsynaptic and glial compartments, have modulatory roles and influence NMDA receptor function. Molecular studies in animal models of schizophrenia (3) and human post mortem brain tissue (4) support the hypothesis that reduced signaling at the postsynaptic NMDA receptor is linked to schizophrenia. The most convincing evidence of mGluR2 and mGluR3 involvement in schizophrenia comes from a recent clinical trial with LY2140023, a novel mGluR2/3 agonist, which demonstrates potent antipsychotic activity in schizophrenia (5).

Since LY2140023 is an agonist at both mGluR2 and mGluR3 receptors, it is not known whether the antipsychotic effects of this drug are mediated via mGluR2, mGluR3 or both receptors. Studies using knockout mice for mGluR2 and mGluR3 suggest that mGluR3 receptors are critical for the neuroprotective effects of mGluR2/3 agonists (6) while mGluR2 receptors were found to have the opposite effect potentiating toxicity. The respective roles of mGluR2 and mGluR3 in animal models of psychosis are still unclear (7). The determination of mGluR2 and 3 expression in high quality human post mortem brain tissue from cases with schizophrenia could localize alterations in mGluR2 and/or mGluR3 and suggest a site of action for the mGluR2/3 agonist.

The endogenous neuropeptide, NAAG, the substrate of GCPII, is prevalent and widely distributed in the mammalian brain (8). It is a selective agonist for mGluR3 and may also be a weak agonist at the NMDA receptor depending on cell type and NMDA receptor subunit composition (8). NAAG is released into the synapse and metabolized by astrocytic GCPII to N-acetyl aspartate (NAA) and glutamate. GCPII inhibition, presumably by increasing NAAG, reverses PCP-induced behaviors in rodents (9).

Previous studies examining the combined expression of mGluR2/3 protein expression in schizophrenia report either no change (10) or an increase in the PFC (11). In this study, we determined the individual expression of mGluR2, mGluR3 and GCPII in the dorsolateral prefrontal cortex (DLPFC), temporal lobe (TC) and motor cortex (MC) from 15 pairs of matched cases of schizophrenia and control human post mortem brain tissue samples.

Materials and Methods

Antibodies—The GCPII antibody was kindly provided by Drs. Joseph Neale and Tomasz Bzdega, Georgetown University. mGluR2- and mGluR3-specific antibodies were obtained from Abcam (Cambridge, MA). The mGluR2 antibody (cat#ab52176) is an affinity-purified antibody produced against an epitope-specific immunogen. Its specificity has been tested by western blot analysis and preadsorption studies. The mGluR3 antibody was generated using a synthetic human peptide predicted not to cross react with mGluR2. Western blot, preadsorption and co-immunoprecipitation studies using mGluR2 and mGluR3 chimeras demonstrate specificity of this antibody for mGluR3 (12). The antibody against beta tubulin (cat#MAB3408) is from Millipore (Billerica, MA) and the horseradish peroxidase coupled secondary antibodies are from Vector Laboratories (Burlingame, CA).

Human postmortem tissue

Characteristics of cases—Human brain tissue was obtained by the Dallas Brain Collection (13). Tissue was collected after obtaining consent from the next of kin along with permission to obtain medical records and to hold a direct telephone interview with a primary caregiver. All clinical information obtained for each case was reviewed by three research psychiatrists and diagnoses made using DSMIV criteria. Blood toxicology screens for drugs of abuse, alcohol and prescription drugs including psychotropics were conducted on each

case. Cases with known history of neurological disorders or axis I psychiatric disorders other than schizophrenia were excluded. The collection of human brain tissue was approved by the Institutional Review Board of University of Texas Southwestern Medical Center. The tissue cohort consisted of 15 pairs of cases of schizophrenia and matched controls (Table 1, individual case demographics in supplement) matched as closely as possible for age, brain pH, postmortem interval (PMI) and RIN (RNA integrity number). 4 additional normal control cases were used to determine the regional distribution of mGluR2 and mGluR3 in the human brain (demographics in supplement).

Tissue Preparation—At the time of brain dissection, tissue samples including the whole cortical gray matter mantle were taken from the DLPFC (BA9), anterior pole of the temporal (BA38), anterior cingulate (BA24), orbitofrontal (BA11), parietal (BA7), occipital cortex (BA19) and motor cortex (BA4). Samples were also dissected from the nucleus accumbens (NAc), caudate nucleus (CN), posterior thalamus (pulvinar), hippocampus and cerebellum. Samples were removed, frozen immediately in a mixture of dry ice and isopentane (1:1,v:v) and at -80°C stored until further use.

Western immunoblotting with scanning densitometry—Tissue samples were removed from -80°C , pulverized on dry ice and homogenized in buffer (1×PBS containing 1%SDS, 1mM PMSF, 20mg/ml leupeptin, 10mg/ml pepstatinA, 2mg/ml aprotinin. 20μg protein per sample was loaded in duplicate on a 10% polyacrylamide gel, transferred to nitrocellulose membrane, blocked for 30min at room temperature (RT) (5% non-fat dry milk, 0.1% Tween20, 50mM Tris-buffered saline; TBS, pH7.5), then incubated overnight at 4°C with mGluR2(1:500 dilution), mGluR3(1:750) or GCP II(1:20) antibody. After washing, blots were incubated with secondary antibody (1:10,000 dilution, goat anti-rabbit IgG) for 30min. Immunoreactive proteins were detected using enhanced chemiluminescence (Amersham, NJ) using Fuji film (Light Labs Company, Dusseldorf, Germany). Film-based images of immunoblots were scanned and immunopositive bands quantified using ImageQuant software (Amersham, UK) blind to diagnosis. β tubulin was used as a loading control.

pH and RIN Determination—These were carried out as previously described (13). Averages and ranges of tissue quality measures are provided for all cases (Table 1; supplement).

Effects of chronic antipsychotic treatment on GCP II and mGluR3 expression—To determine potential effects of chronic antipsychotic treatment on GCP II and mGluR3, Sprague Dawley rats (Charles River, Wilmington, MA) were treated with a first generation (haloperidol), second generation (risperidone) antipsychotic or placebo (n=10 each) in drinking water continuously for 6 months as previously described (14). Rats were then sacrificed, trunk blood collected and plasma frozen at -20°C until analysis. Drug levels were measured in the laboratory of Dr Tom Cooper as previously described (14). Rodent brains were rapidly removed, the frontal cortex dissected and frozen by immersion in -40°C isopentane.

Statistical Analysis

The demographic variables pH, PMI, RIN and age were compared between groups using *t*-tests. Correlations between protein levels with age, RIN and PMI were run with a Spearman Rank Order correlation. The effect of diagnosis on each protein species was analyzed using a mixed model ANOVA with diagnosis as the between group factor and brain area (DLPFC, TC, MC) as the within group factor. Significant findings were further analyzed using *post hoc t*-tests. When a correlation was found between a demographic variable and protein

levels, analysis of covariance (ANCOVA) was performed to correct for the confounding variable. All statistical tests were two-tailed, p values $< .05$ were considered statistically significant. Values outside 2 S.D.s from the mean were considered outliers and not included in the statistical analyses.

Results

Regional distribution of mGluR2 and 3 protein expression in humans

Four normal cases (age 32.5 ± 9.7 , PMI 19.5 ± 5.3 , RIN 8.35 ± 0.61) were used to determine the regional distribution of mGluR2 and mGluR3 in the brain (fig 1). High levels of expression were observed in the DLPFC, anterior cingulate, orbitofrontal, parietal and occipital cortex. Lower levels were detected in the caudate nucleus, nucleus accumbens and cerebellum with lowest levels in the thalamus. The hippocampus expressed relatively high levels of mGluR2. The expression of mGluR3, in general, was more evenly distributed within cortical regions and the hippocampus expressed highest levels of mGluR3. In contrast to mGluR2, high levels of mGluR3 were seen in the caudate nucleus and nucleus accumbens with moderate levels in the thalamus and cerebellum. Due to the relatively high expression of both mGluR2 and mGluR3 in the DLPFC and TC, regions consistently implicated in the pathophysiology of schizophrenia, we selected these brain regions and a putative control region (MC) to determine differences in protein expression in schizophrenia compared to control cases.

Comparison between controls and cases of schizophrenia

15 pairs of cases with schizophrenia and normal controls were used in the analysis of GCP2, mGluR2 and mGluR3 protein expression in the DLPFC, TC and MC. There were no significant differences in diagnostic groups with respect to age ($df_{1,28}, p=0.86$), PMI ($df_{1,28}, p=0.26$), pH ($df_{1,28}, p=0.6$) or RIN ($df_{1,28}, p=0.37$). No significant correlations were obtained between mGluR2 or mGluR3 with any demographic variable (all R between -0.13 and 0.33 , all $p > 0.18$) except between mGluR2 and RIN in the DLPFC ($R=0.56, p=0.01$). GCP2 levels showed a significant correlation with PMI in the DLPFC ($R=0.37, p=0.04$) and with pH ($R=0.61, p<0.001$) in the TC.

GCP2—While there was no main effect of diagnosis on GCP2 ($F=0.08, df_{1,27}, p=0.77$), an effect of region ($F=17.65, df_{1,54}, p<0.0001$) and an interaction between diagnosis and region ($F=4.11, df_{1,52}, p=0.013$) were found. Post hoc analysis showed that GCP2 protein was significantly higher in the DLPFC in schizophrenia (Fig 2; $t=2.27, df_{1,26}, p=0.03$). There were 2 pairs of cases (C9/S9, C11/S11) with low RIN levels. An ANCOVA covarying for RIN, PMI and pH did not change the significance of these results ($F=3.42, df_{3,22}, p=0.035$).

mGluR3—While there was no main effect of diagnosis on mGluR3 ($F=3.2, df_{1,21}, p=0.08$), an effect of region ($F=186.1, df_{1,42}, p<0.0001$) and an interaction between diagnosis and region ($F=4.82, df_{1,52}, p=0.013$) was found. Post hoc tests showed that mGluR3 protein was significantly lower in the PFC in schizophrenia (Fig 2; $t=2.49, df_{1,22}, p=0.02$). Analysis without C9/S9 and C11/S11 (cases with low RIN) did not change the significance of the results ($t=2.55, df_{1,19}, p=0.019$).

mGluR2—There was no main effect of diagnosis on mGluR2 ($F=1.27, df_{1,27}, p=0.27$) but an effect of region ($F=47.1, df_{1,54}, p<0.0001$). No significant interaction was seen between diagnosis and region ($F=1.91, df_{1,54}, p=0.16$). Since correlations were seen between mGluR2 protein levels and RIN, an ANOVA covarying for RIN was performed. The interaction between diagnosis and region remained non-significant ($F=1.55, df_{3,23}, p=0.22$). Exploratory

analyses showed no significant differences in mGluR2 expression between groups in any of the 3 regions (supplement).

Effects of chronic antipsychotic treatment on GCPII and mGluR3 expression

The question of whether the differences in protein expression could be associated with chronic antipsychotic drug (APD) treatment was addressed in two different ways. In the first approach, rats were treated with two different APDs chronically, using doses that produce plasma levels in the human therapeutic range. Tissue from the frontal cortex was taken from 6 month APD-treated rats and analyzed for GCPII and mGluR3 protein expression. Haloperidol and risperidone+risperidone-9OH levels in plasma from trunk blood collected at time of decapitation (n=10 each) were 6.8 ± 1.1 ng/ml and 6.2 ± 3.2 ng/ml respectively, demonstrating adequate dosing in these animals. There was no effect of antipsychotic treatment on GCPII protein ($F=1.36, df_{1,27}, p=0.27$) or mGluR3 protein levels ($F=1.48, df_{1,25}, p=0.24$) in the frontal cortex compared to control tissue (Fig 3).

In addition, differences in protein levels between cases of schizophrenia on antipsychotic medication at the time of death (n=10) were contrasted with cases off antipsychotic medication at the time of death (n=5). Although the number of off-drug cases is low, the results support the lack of an APD effect on these proteins in the PFC (GCPII, $t=1.62, df_{1,13}, p=0.13$; mGluR3, $t=0.66, df_{1,10}, p=0.52$; figure in supplement).

Discussion

The individual expression profiles of mGluR2 and mGluR3 protein in the human brain have not been previously demonstrated because selective antibodies were lacking. The recent availability of antibodies specific for each of these receptors has made it possible to determine their individual expression in the normal human brain and disease populations. In this study, we examined the expression profile of these proteins across a range of brain regions in the normal human brain. Then we compared tissue samples from cases of schizophrenia and matched controls in two brain regions important to the pathophysiology of schizophrenia, the DLPFC and TC, and a putative control region, MC. These studies show that mGluR3 and GCPII, are altered in the DLPFC, but not TC or MC, in schizophrenia.

mGluR2 and mGluR3 have distinct expression patterns in the human brain. Levels and distribution of mGluR2 and mGluR3 in human cortical regions is similar to that seen in laboratory animals (15),(16). In contrast, the human NAc and CN express relatively low levels of mGluR2 and moderate levels of mGluR3 protein compared to high levels seen in the rodent. The lower expression of mGluR2 and 3 in the NAC and CN, regions that receive projections from dopamine neurons, may be important in light of the modulatory roles of these receptors on dopamine neurotransmission. We previously reported that mGluR2 and 3 mRNA are expressed in dopamine neurons (17).

Differences in schizophrenia

Several findings stand out in this study. First, the changes in mGluR3 and GCP II protein expression in schizophrenia were localized to the DLPFC (BA9), a brain region repeatedly implicated in this illness (18,19). Many studies have shown that volunteers with schizophrenia perform poorly on working memory tasks and show deficits in DLPFC functional activation (18,19). Moreover, molecular studies have identified several replicated alterations in the DLPFC (20). Our findings are consistent with the localization of molecular alterations to this brain region and extend previous knowledge to the mGluR system. Secondly, we find a reduction in mGluR3, not mGluR2, leading us to propose that mGluR3

could be the specific molecular target of the novel mGluR2/3 agonist LY2140023 mediating its antipsychotic action. Moreover, both mGluR3 and GCPII are significantly changed in the same brain region suggesting a potential association between these two proteins which are already known to be functionally related. NAAG, an endogenous agonist of mGluR3, is the substrate of GCPII. Levels of NAAG are largely determined by GCPII activity, such that GCPII activity may be considered an indirect marker of NAAG levels; increased GCPII levels indicate low NAAG concentrations (8). Based on this, we reason that NAAG levels are decreased in the DLPFC in schizophrenia. These changes are consistent with PCP studies which show that GCPII inhibition reduces PCP-induced behaviors in laboratory animals (21). Thirdly, we have provided evidence in this paper that the changes in GCP II and mGluR3 expression are not likely to be secondary to antipsychotic treatment.

Previous studies of GCP II and mGluR3

GCPII levels and activity in the schizophrenia PFC have been previously examined but with inconsistent results; some report no change in mRNA expression (22), others, a decrease in quantitative binding (23), still others reduced enzyme activity (24). While these studies examined GCPII in BA46, in this report, we used tissue from BA9. In this study, we report increases in protein expression, not in mRNA levels or enzyme activity. Of note, we find an influence of PMI on GCPII levels, a variable that may influence results across all laboratories. Further, disease heterogeneity in schizophrenia always has to be considered.

Expression profiles of mGluR2 and 3 in the DLPFC in schizophrenia have also been previously examined (10,11,17,25,26) but with inconsistent results. At the mRNA level, no changes in mGluR3 in the DLPFC have been reported (17,25) although alterations in expression of a splice variant may exist (26). Crook et al, did not find any change in the combined expression of mGluR2/3 protein in BA46, while a later study (11) reported an increase in mGluR2/3 immunoreactivity in BA46 but not in BA9. Corti et al (27) developed a mGluR3-specific antibody and tested it on BA10 schizophrenia tissue but found no change in total or monomeric form of mGluR3. They did find a decrease in the dimeric form of mGluR3, thought to be the inactive form (28). This paper reports reduced expression of monomeric mGluR3 protein in BA9 in schizophrenia. The lack of effect of diagnosis on mGluR2 expression reflects normal levels of mGluR2 protein and is consistent with our previous study showing unchanged levels of mGluR2 mRNA in the grey matter of the DLPFC in schizophrenia (17) but differs from a recent report in small cohort of 7 pairs of medication-free cases of schizophrenia that found reduced mGluR2 mRNA (12). In this current study, no difference in schizophrenia cases on-drug vs. off-drug cases exist, however due to the small sample size, further examination may be warranted. We did not find an effect of antipsychotic treatment on mGluR3 or GCPII protein expression, an outcome consistent with previous studies (26,29).

Functional role of mGluR3 receptors

mGluR3 receptors are located at the presynaptic terminal, postsynaptic terminal and glial cells (30) and have distinct functions at each site. Overall, activation of mGluR3 receptors has been linked to the modulation of a variety of physiological functions including release of neurotransmitters, regulation of synaptic plasticity and enhancing neuroprotection (8). Below, we describe a few mGluR3-mediated effects that may be of particular significance in schizophrenia.

Neuronal mGluR3 receptors modulate the glutamate and gamma-amino butyric acid (GABA) systems, both implicated in the pathophysiology of schizophrenia (8). Presynaptic mGluR3 receptors inhibit the release of glutamate and GABA into the synapse, while post synaptic mGluR3 receptors have been shown to modulate NMDA and GABA-A receptor

function (8). In the PFC, the enhancement of NMDA receptor function by mGluR2/3 receptors (31), most likely mGluR3 (9), has been proposed as the physiological basis for reversal of PCP-induced behaviors (9,31). Activation of mGluR3 receptors also regulate GABA-A receptor subunit expression, specifically $\alpha 1$, $\alpha 5$, $\alpha 6$, $\beta 2$, $\gamma 2$, δ subunit (32), S.G. unpublished data). In the schizophrenia PFC, alterations in several GABA-A subunits, including $\alpha 1$, $\gamma 2$ and δ , have been reported (33). It is plausible that mGluR3 decreases in DLPFC reported in this paper are related to the GABA-A abnormalities in schizophrenia.

Astrocytic mGluR3 receptor activation generates the formation of transforming growth factor $\beta 1$ (TGF $\beta 1$) and TGF $\beta 2$ (34), factors that are neuroprotective. The TGF β pathway proteins modulate synaptic plasticity (35), glutamatergic function (36) and enhance dendritic growth and spine formation (37). Further, activation of astrocytic mGluR3 receptors play a role in modulating brain microcirculation (38). Baslow et al. suggest that NAAG activates astrocytic mGluR3, triggers Ca^{2+} currents that spread to astrocytic endfeet in contact with the vascular system, where a secondary release of vasoactive agents increase blood flow. Based on this, we speculate that low NAAG levels are associated with the low levels of regional blood flow often reported in PFC in schizophrenia (39).

Model of mGluR3 dysfunction in the DLPFC in schizophrenia

We propose a formulation of glutamatergic function in the schizophrenia PFC that recognizes the complex roles of mGluR3 in brain function and is consistent with a putative glutamate reduction in schizophrenia (fig4a). At the postsynaptic mGluR3 receptor, the reduction in protein would diminish glutamate signal through a loss of positive modulation at the NMDA receptor (fig 4B). Moreover, at the glial mGluR3, reduced receptor activation would decrease TGF β release. The reduced level of this growth factor may be associated with the smaller size of prefrontal neurons and reduction in dendritic branching reported in schizophrenia. Further, reduced astrocytic mGluR3 activation can lead to a decrease in Ca^{2+} signaling and subsequent reduction in local blood flow in DLPFC in schizophrenia demonstrated by numerous previous studies in schizophrenia (39). In addition, the increase in GCPII on glial cells decreases NAAG levels through active degradation, further reducing mGluR3-mediated signaling.

We propose that activation of mGluR3 by agonists, like LY2140023, would reverse the changes brought about by the reduction in mGluR3 in schizophrenia PFC (Fig 4C). While mGluR3 activation would further reduce NAAG release via presynaptic inhibition, it would generate an increased response at the postsynaptic mGluR3 receptor to potentiate NMDA function (fig 4C). The net effect would be an increase in NMDA-mediated signaling at the glutamate synapse. In addition, disease-associated GABA deficits mediated by low mGluR3 would also be reversed by LY2140023. In the glial compartment, mGluR3 activation would augment TGF β levels, reversing the potentially deleterious effects on neuronal morphology and function. Further, glial mGluR3 activation could restore local blood flow in the PFC.

To summarize, LY2140023 could reverse mGluR3-mediated deficits in schizophrenia to increase function at the NMDA receptor, 'normalize' GABA function, increase TGF β and restore DLPFC perfusion. These interpretations provide a model of prefrontal cortical dysfunction in schizophrenia in which to conceptualize our data and postulate mechanisms for the action of the selective mGluR2/3 agonist.

Limitations of this study

The extent to which human postmortem tissue reflects *in vivo* conditions is always a question, but is buttressed in this study by the selecting of high tissue quality characteristics. Several parameters have been identified to mark tissue quality, such as RIN and PMI (13).

The tissue used in this study was of high quality judged by these parameters. Moreover, the potential effects of antemortem antipsychotic treatment on gene expression products can be an important potential confound. While this study attempted to address the latter issue using two approaches, and both suggested no chronic medication effect, the possibility of a drug effect must always be considered. Also, one cannot exclude the possibility that these drugs have distinct effects in human compared to rodent brain. In this study, we examined protein levels in the gray matter of the cortical regions. The possibility of changes in the DLPFC white matter (17) will need further evaluation. In addition, we cannot comment on whether the mGluR3 change we find localizes to any particular receptor population (i.e. presynaptic, postsynaptic or glial) nor can we draw conclusions about the dynamic regulation between GCPII and mGluR3. These questions will be addressed in future studies.

In closing, we provide evidence that NAAG-mediated neurotransmission at the mGluR3 receptor is disrupted in the DLPFC in schizophrenia based on human post mortem tissue measures of the proteins involved. The defects we report could be attenuated by mGluR3 agonists reversing the consequences of the protein changes, putatively ameliorating the symptoms of the illness. This leads us to speculate that this molecular target could mediate the therapeutic response to LY2140023, the first mGluR2/3 agonist with an antipsychotic action in schizophrenia (5).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Distribution in the normal brain

Fig. 1.

Bar graphs showing levels of mGluR2 and mGluR3 in 10 brain regions (PFC = dorsolateral prefrontal cortex, ACC = anterior cingulate, OFC = orbitofrontal cortex, PC = parietal cortex, CN = caudate nucleus, NAc = nucleus accumbens, TH = thalamus, HC = hippocampus, CB = cerebellum, OCC = occipital cortex). Grey bars represent mGluR2 protein levels, black bars represent mGluR3 protein. Data shown are mean $\pm$ standard error.

Fig. 2.

Post mortem human tissue samples from 15 pairs of matched controls (CON) and cases of schizophrenia (SCH) were analyzed by immunoblotting to detect GCPII, mGluR2 and mGluR3 protein levels relative to β tubulin (loading control) in the prefrontal cortex (PFC), temporal cortex (TC) and motor cortex (MC) in cases. Data shown for GCP II and mGluR3 are mean $\pm$ S.D. Asterisk (*) denotes significant difference between diagnostic groups ($p < 0.05$)

Effect of antipsychotics on GCP II and mGluR3 **Fig. 3.**

Graphs showing absence of an effect of chronic treatment in rodents ($n = 10$ each group) with haloperidol (1.5 mg/kg/day; hatched bars) or risperidone (6 mg/kg/day; filled bars) compared to water on GCP II and mGluR3 protein expression in the frontal cortex. Data shown are mean $\pm$ S.D.

Fig. 4.

Schematic representation of the 3 compartments of the normal synapse (pre-synaptic, post synaptic terminals and glial cell) with localization of mGluR3 and GCP II (A). NAAG is released from the pre-synaptic terminal into the synapse (1) where it can activate mGluR3 receptors located at (a) the postsynaptic terminal to potentiate NMDA responses (+) and regulate GABA-A receptor subunit expression, (b) presynaptic terminal to regulate its own release (–) or (c) glial cell where it mediates the release of TGF β and modulates local microcirculation. In schizophrenia (B), high GCPII and low mGluR3 impair NAAG-mediated activation at the mGluR3 receptor. This could reduce NMDA potentiation, alter GABA-A receptor composition, release NAAG which is metabolized by higher GCPII levels, decrease TGF β release and reduce regional blood flow. (C) mGluR2/3 agonists could reverse the effects of high GCPII and low mGluR3 by restoring function at the mGluR3 receptor.

Table 1

Summary of demographic and tissue quality measures of 15 pairs of controls and cases of schizophrenia used in the determination of GCP II, mGluR2 and mGluR3 protein expression. PMI = postmortem interval, RIN = RNA integrity number. Av = average, S.D. = standard deviation.

n = 15 each	Age (years + S.D.)	PMI (hours + S.D.)	RIN (+ S.D.)	pH (+ S.D.)
CONTROL	50.4 + 16.1	16.5 + 8.4	7.5 + 2.1	6.7 + 0.3
SCHIZOPHRENIA	49.3 + 17.8	19.5 + 8.16	7.2 + 2.2	6.6 + 0.3