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Glutamatergic dysfunction linked to energy and membrane lipid metabolism in frontal and anterior cingulate cortices of never treated first-episode schizophrenia patients

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ABSTRACT

Background: Glutamatergic dysfunction and altered membrane lipid and energy metabolism have been repeatedly demonstrated in the frontal/prefrontal and anterior cingulate cortex (ACC) in schizophrenia. Though having been already studied in animals, the presumed link between glutamatergic function and structural plasticity has not been investigated directly in the human brain yet. We measured glutamate (Glu), focal energy metabolism, and membrane phospholipid turnover to investigate main pathologies in those key brain regions of schizophrenia.
Methods: ^{1}H- and ^{31}P-Chemical Shift Imaging (CSI) was combined in a single session to assess Glu and markers of energy (PCr, ATP) and membrane lipid (PME, PDE) metabolism in 31 neuroleptic-naïve first acute onset psychosis patients and 31 matched healthy controls. Multivariate analyses of covariance were used to assess disease effects on Glu and to investigate the impact of Glu alterations on phospholipid and energy metabolites.
Results: Glu levels of patients were increased in the frontal and prefrontal cortex bilaterally and in the ACC. Higher Glu was associated with increased left frontal/prefrontal PME and right frontal/prefrontal PDE in patients, which was not observed in healthy controls. In contrast, higher Glu levels were associated with lower PCr or ATP values in the frontal/prefrontal cortex bilaterally and in the right ACC of controls. This was not observed in the right ACC and left frontal/prefrontal cortex of patients.
Conclusion: Frontal glutamatergic hyperactivity is disconnected from physiologically regulated energy metabolism and is associated with increased membrane breakdown in right and increased membrane restoration in left frontal and prefrontal cortical regions. As indicated by previous findings, this pathology is likely dynamic during the course of first acute illness and possibly associated with negative symptoms and cognitive impairment. Our findings underline the importance of further research on neuroprotective treatment options during the early acute or even better for the ultra-high risk state of psychotic illness.

1. Introduction

In this work, we follow two lines of evidence concerning the pathogenesis of schizophrenia. First, we consider the n-methyl-d-aspartate (NMDA) glutamate receptor dysfunction hypothesis, which pertains to biological correlates of hypofrontality (Coyle, 2004; Kantrowitz and Javitt, 2012; Olney et al., 1999). Secondly, we consider the membrane lipid hypothesis (Horrobin et al., 1994) that assumes deficient generation or excessive breakdown of membrane phospholipids as a causative pathology in schizophrenia, and that is based on evidence of disturbed membrane lipid turnover in specific brain regions of schizophrenic patients. Both hypotheses have been related to disturbances of neurodevelopment, neuronal and synaptic plasticity as well as to hyperexcitation, excitotoxicity and oxidative stress (Farooqui et al., 2007; Horrobin, 1998; Keshavan, 1999; Michaelis, 1998).

Glutamate (Glu) is the excitatory neurotransmitter of pyramidal cells and is present in about 40% of the brain's synapses. It plays a crucial

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role in cortical projections to diverse subcortical regions (hippocampus, amygdala, basal ganglia), in inter-cortical projections and in thalamo-cortical tracts, such as the glutamatergic connections between thalamus and medial prefrontal and anterior cingulate cortices. In addition, corticofugal glutamatergic neurons control monoaminergic neurons and provide direct activation of dopaminergic, noradrenergic and serotonergic neurons. Furthermore, they inhibit monoaminergic neurons via activation of intermediary GABA-ergic inter-neurons enabling highly sensitive regulation of monoamines (Vogt, 1993).

In animal studies, the different regulation states of the glutamate/dopamine system were found to be important for early neurodevelopment and synaptic plasticity during adolescence/early adulthood (Bondi et al., 2012). Accordingly, regulation deficits were identified to predispose disturbed synaptic plasticity in functionally important brain regions leading to the manifestation of symptoms summarized as "hypofrontality" in humans (Grace, 1993; Yin et al., 2012). Excess dopaminergic activity can enhance release of Glu, which in turn may cause excitotoxic cell or dendrite damage (Finlay and Zigmond, 1997; Reid et al., 1997) with crucial consequences in the adolescent/young adult brain.

Combining the notions of glutamatergic dysfunction, dysregulation of brain activity, and impaired membrane phospholipid turnover, we hypothesize that disease related glutamatergic dysfunction is linked to altered energy and membrane metabolism. However, so far it has been difficult to demonstrate this association in the human brain. Therefore, we investigated this link by means of combined proton and phosphorous MR spectroscopic ($^{1}H/^{31}P$-MRS) measurements.

In vivo proton spectroscopy (^{1}H-MRS) allows quantification of several brain metabolites, including Glu. Glu is synthesized in axon terminals of glutamatergic neurons, either from α-ketoglutarate or from glutamine (Gln). It has been estimated that 80% of stimulus-released Glu is derived from Gln (Erecinska and Silver, 1990; Ross, 1991). A dynamic balance exists between Glu and Gln to maintain appropriate levels of Glu (Erecinska and Silver, 1990; Williamson et al., 1996).

A recent meta-analysis of ^{1}H-MRS studies that focused on the Glu/Gln complex revealed decreased medial frontal Glu and increased Gln in first-episode schizophrenia patients (FEP) (Marsman et al., 2013). Reviewing findings in never-medicated and medicated patients separately, drug-naïve patients were reported with no changes of glutamatergic levels (measured by Glx, Gln) in the dorsolateral prefrontal cortex, but elevated glutamatergic levels in the medial prefrontal and anterior cingulate cortex (ACC) (Poels et al., 2014). Single studies reported also increased glutamatergic levels in medial temporal lobe structures and the hippocampus (Glx) (Kraguljac et al., 2013) as well as in the associative striatum and cerebellum (Glx and Glu) (de la Fuente-Sandoval et al., 2013). Findings of glutamatergic levels in the thalamus are still heterogeneous with some studies showing an increase in never treated patients (Aoyama et al., 2011; Theberge et al., 2002, 2007). There is also some uncertainty on the interpretation of glutamatergic alterations. While some authors interpreted increased (medial frontal) Gln as an expression of a Glu deficit due to disturbed Glu/Gln cycling (high Gln associated with low Glu (Bartha et al., 1997)), others ascribed the increased Gln level (e.g., in the ACC) to an increased glutamatergic activity (Theberge et al., 2002).

^{31}P-MRS, on the other hand, makes it possible to assess phospholipid metabolites (phosphomonoesters, PME, precursors in phospholipid synthesis and membrane restoration; phosphodiesters, PDE, metabolites of phospholipid degradation and indicator of focal membrane damage) and high-energy phosphates (adenosine triphosphate, ATP, main energy transferring compound; phosphocreatine, PCr, interim storage of excess energy). In schizophrenia research, the main findings of ^{31}P-MRS studies include phospholipid alterations in prefrontal, anterior cingulate cortices, thalamus, insular cortex, basal ganglia (e.g., caudate nucleus) and anterior cerebellum (Gangadhar et al., 2004; Jayakumar et al., 2003; Jensen et al., 2004; Smesny et al., 2007; Volz et al., 2000). In never treated patients, the most consistent findings were observed in prefrontal and frontal brain regions, namely decrease of PME and/or increase in PDE as well as decrease in ATP and/or increase in PCr (Keshavan et al., 2000). These findings were interpreted as expression of focally increased neuronal/synaptic membrane damage accompanied by deficits in maintaining or re-establishing physiological membrane texture and energy supply (Smesny et al., 2007).

In this study, combined $^{1}H/^{31}P$-MRS was performed in cohorts of never treated FEP and matched healthy controls (HC) to address the following hypotheses:

1. Glu is dysregulated in the frontal/prefrontal and anterior cingulate cortex indicating glutamatergic dysfunction. If verified, this finding serves as the precondition to investigate the following main hypothesis.
2. In the frontal/prefrontal and anterior cingulate cortex of patients altered glutamatergic function is associated with focally altered PDE and PME and/or altered PCr and ATP.

2. Methods

2.1. Subjects

We investigated 31 FEP (16 males/15 females) and 31 HC (16 males/15 females), all right-handed and matched for age and gender (Table 1). All patients suffered their first schizophrenic episode, paranoid-hallucinatory sub-type, according to DSM-IV criteria for schizophrenia. All diagnoses were independently established by two board certified psychiatrists (S.Sm., H.S.) and confirmed by standardized structured clinical interviews (SCID-IV) (Wittchen et al., 1997). Psychopathology

Table 1
Group demographics and psychopathological measures. All patients were recruited between 2009 and 2013, either at the time of first hospital admission or when seeking help in the outpatient psychosis prevention and early intervention clinic. Due to this clinical setting, patients were included shortly before or when just meeting the DSM-IV time criterion (see also duration of untreated psychosis DUP) and clinical criteria for a schizophrenic episode. At the time of measurement 28 patients fulfilled the criteria of paranoid-hallucinatory schizophrenia (incl. time criterion) and 3 patients the DSM-IV criteria of schizophreniform disorder. Regular follow-up interviews confirmed meanwhile diagnosis of paranoid-hallucinatory schizophrenia also in the latter three patients.
Additionally to interview questions about substance use in all participants, FEPs additionally underwent a breath alcohol concentration test and a screening blood and urine test for illegal substances (among others amphetamines, cocaine, morphine and its derivates). None of these screening tests in FEP revealed a positive result at the time of hospital admission.

	First episode patients (FEP)	Healthy controls (HC)
N	31	31
Gender (male)	16 (52%)	15 (48%)
Age (years) mean (±SD)	25.97 (±4.95)	25.42 (±5.18)
Alcohol		
Less than weekly	6 (19.4%)	20 (64.5%)
1–6 drinks/week	23 (74.2%)	10 (32.3%)
Daily	2 (6.4%)	1 (3.2%)
Marijuana		
No	10 (32.3%)	18 (58.1%)
Less or equal 2 g/week	18 (58.1%)	13 (41.9%)
More than 2 g/week	3 (9.7%)	0 (0.0%)
Psychiatric medication		
Antipsychotic medication	Naïve	
Antidepressant	0	0
Benzodiazepine (sporadic)	6 (19.4%)	0
Duration of untreated psychosis (DUP)	5.2 ± 1.7 month	
Psychopathology		
PANSS total score	58.7 (±7.7)	
PANSS positive subscale	31.8 (±3.5)	
PANSS negative subscale	27.5 (±9.2)	
PANSS global subscale	41.7 (±8.1)	

Data are mean (±standard deviation, SD) or n (%). PANSS denotes Positive and Negative Syndrome Scale.

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