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Significance of NMDA receptor-related glutamatergic amino acid levels in peripheral blood of patients with schizophrenia

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ABSTRACT

Hypo-function of *N*-methyl D-aspartate (NMDA) receptors is strongly involved in the brain pathophysiology of schizophrenia. Several excitatory amino acids, such as endogenous glutamate, glycine, serine and alanine, which are involved in glutamate neurotransmission via NMDA receptors, were studied to further understand the pathophysiology of schizophrenia and to find a biological marker for this disease, particularly in peripheral blood. In this literature review, we connect several earlier clinical studies and several studies of excitatory amino acid levels in peripheral blood in a historical context. Finally, we join these results and our previous studies, the Juntendo University Schizophrenia Projects (JUSP), which investigated plasma glutamatergic amino acid levels in detail, and considered whether these amino acid levels may be diagnostic, therapeutic, or symptomatic biological markers. This review concludes that peripheral blood levels of endogenous glycine and alanine could be a symptomatic marker in schizophrenia, while peripheral blood levels of exogenous glycine and alanine in augmentation therapies could be therapeutic markers. Noteworthy peripheral blood levels of endogenous D-serine could reflect its brain levels, and may prove to be a useful diagnostic and therapeutic marker in schizophrenia. In addition, measurements of new endogenous molecules, such as glutathione, are promising. Finally, for future therapies with glutamatergic agents still being examined in animal studies, the results of these biological marker studies may lay the foundation for the development of next-generation antipsychotics.

1. Introduction

Similar to hyper-dopaminergic neurotransmission, disrupted glutamatergic neurotransmission has been suggested to be one of the mechanisms of brain pathophysiology in schizophrenia. In particular, hypo-function of *N*-methyl D-aspartate (NMDA) receptors is strongly involved in brain pathophysiology (Krebs, 1995; Olney and Farber, 1995); over half a century ago, abuse of phencyclidine (PCP), an antagonist of NMDA glutamate receptors, was shown to induce schizophrenic-like symptoms (Luby et al., 1959). Thereafter, clinical studies of PCP-induced schizophrenic-like symptoms were reported (Halberstadt, 1995; Javitt and Zukin, 1991), and animals treated with PCP show similar cognitive dysfunctions involved in the main pathophysiology of schizophrenia (Andersen and Pouzet, 2004; Halberstadt, 1995; Hashimoto et al., 2008; Javitt, 1987; Saransaari et al., 1993). Following these studies, a disrupted glutamatergic neurotransmission in schizophrenic brains was seen in postmortem brain studies (Akbarian et al., 1996; Deakin et al., 1989; Nishikawa et al., 1983; Ohnuma et al., 1998, 2000a,b, 2003, 2005). While some studies failed to reproduce these findings and instead showed negative findings, most speculated that disturbed glutamatergic neurotransmission exists in schizophrenic brains.

At the present, two possible pathophysiologies could be involved in the glutamate hypothesis of schizophrenia. PCP blockade of NMDA receptor neurotransmission (resulting in hypo-function of NMDA receptors) is considered to be a pathophysiology in glutamate hypothesis, but PCP administration also led to glutamate outflow in the prefrontal cortex in animal studies; thus, this phenomenon may increase a-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)/kainic acid (KA) receptor activity (hyper-function of AMPA/KA receptors), as contrary imbalances between NMDA and other ionotropic glutamate receptors may coexist in the brains of patients with schizophrenia (Moghaddam et al., 1997). Clinical studies have also suggested the coexistence of increased AMPA/KA receptor activity in schizophrenic brains, as lamotrigine, an anticonvulsant that inhibits exocytosis of glutamate from synaptic vesicles (resulting in decreased glutamate release at the synaptic cleft), but improves some schizophrenic symptoms. Taken together, these results indicate that while hypo-function of the NMDA receptor exists, hyper-function of AMPA/KA receptors is also present in schizophrenic brains

Abbreviations: BPRS, Brief Psychiatry Rating Scale; CSF, cerebrospinal fluid; DAO, D-amino acid-oxidase; GLS1 and GLS2, glutaminase 1 and 2; MRS, magnetic resonance spectroscopy; mGluRs, metabotropic glutamate receptors; NMDA, *N*-methyl D-aspartate; PCP, phencyclidine; PPI, prepulse inhibition; SRR, serine racemase.

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(Tiihonen et al., 2009). Although the glutamate hypothesis in schizophrenia remains uncertain, hypo-function of NMDA receptors, which is considered to be the main pathophysiology in the glutamate hypothesis, is supported by several lines of experimental/clinical evidence; thus, the neurotransmitters related to NMDA receptors have been vigorously investigated in schizophrenia. Not only glutamate, but several other excitatory amino acids are involved in glutamate neurotransmission via NMDA receptors. Glycine (a small nonessential amino acid that functions as an obligatory co-agonist at NMDA receptors through its action at a strychnine-insensitive binding site on the NMDA receptor complex), serine (a co-agonist that enhances NMDA receptor activity via binding to the glycineB site), and alanine (an agonist that binds to the glycine site of NMDA receptors) are also considered to be involved in the hypo-function of NMDA receptors in patients with schizophrenia. These amino acids have been studied in detail *in vivo* and *in vitro* to further understand the pathophysiology and identify a biological marker for schizophrenia. In this review, we connect these earlier clinical studies and several studies of excitatory amino acid levels in peripheral blood in a historical context. Finally, we join these results and our previous studies, the Juntendo University Schizophrenia Projects (JUSP), which investigated plasma glutamatergic amino acid levels in detail, and looked at whether levels of these amino acids may be diagnostic, therapeutic, or symptomatic biological markers. We discuss the significance and limitations of these studies, the current status of these investigations, and issues for future study.

2. Methods

A reference search was performed of the Medline (1960–May 2010) and ScienceDirect (January 1960–May 2010) databases. Keywords included schizophrenia, individual glutamatergic amino acid names (glutamate, glycine, serine, alanine, and glutathione), plasma, serum, cerebrospinal fluid, and brain. Studies were included if they satisfied the following criteria: 1) patient populations consisted of only schizophrenia or schizophrenia spectrum disorders (i.e., schizotypal disorders and schizoaffective disorders) including schizophrenia; 2) amino acids were measured by established methods such as high-performance liquid chromatography; and 3) the article was published in English. In addition, references from the selected papers were evaluated and included if they were found to be relevant to the focus of this literature review.

3. Considerations in interpreting the studies of peripheral amino acid levels

In order to determine whether disrupted endogenous glutamatergic neurotransmission could be a biological marker of schizophrenic pathophysiology, several studies were performed more than three decades ago, focusing on living patients with schizophrenia, including a noninvasive, nontechnical and inexpensive examination (including shorter examination times), and measurement of plasma/serum glutamatergic amino acids, which allowed enrollment of a large number of patients (Table 1). However, to interpret these peripheral amino acid studies, several considerations were needed. For example, the exclusion of factors that may affect peripheral amino acid levels and consideration of whether peripheral levels reflect brain levels or only reflect metabolism of these amino acids in other peripheral organs (i.e., liver) were needed.

3.1. Effect of nutrition status

First, it was necessary to consider whether several physical variables, such as age, gender, meals, exercise, nutrition status (body weight, body mass index, plasma total cholesterol, triglyceride, protein), and duration of illness and hospitalization affect peripheral blood amino acid levels. We confirmed that levels of plasma glutamate, glycine, L- and D-serine, and alanine were not affected by these clinical factors in a relatively large number of patients (Hatano et al., 2010; Maeshima et al., 2007; Ohnuma et al., 2008). On several occasions, although patients with exacerbated schizophrenia had appetite loss and excitement (increased calorie consumption) resulting in emaciation, this emaciation did not change the amino acid levels in plasma and cerebrospinal fluid (CSF) (Martinez et al., 1993). However, emaciation may lead to increased glutamate and alanine levels in humans (Martinez et al., 1993). As mentioned below, plasma glutamate and alanine levels in patients with schizophrenia are lower during the acute stage, and levels increase with the remission stage, which may indirectly explain how emaciation in schizophrenia did not affect plasma amino acid levels (Hatano et al., 2010; Maeshima et al., 2007). Nevertheless, a possible effect of nutrition status on plasma and CSF amino acid levels can easily be excluded by collecting samples before breakfast and exercise in all patients, and this was usually done in the previous studies.

3.2. Effects of antipsychotics

An important consideration in studies comparing patients taking antipsychotics and normal unmedicated individuals is whether observed changes in peripheral amino acid levels reflect the pathophysiology of the disease itself or are due to direct effects of antipsychotic medication (type and dosage). Detailed exclusion of the effects of antipsychotics from clinical studies is indispensable in interpreting observed significant changes in peripheral amino acid levels in schizophrenia. Although one cannot draw firm conclusions about whether observed amino acid changes in schizophrenia reflect the effects of antipsychotics or the status of schizophrenia, the dosage of antipsychotics did not affect serum D-serine levels in schizophrenic patients (Hashimoto et al., 2003; Tsai et al., 1999). Our previous study also showed no correlations between changes in the levels of the investigated amino acids and the dosage of antipsychotics (Hatano et al., 2010; Maeshima et al., 2007; Ohnuma et al., 2008). Furthermore, neither conventional nor atypical antipsychotics changed brain D-serine levels in a rat brain study (Sakurai et al., 2004), and atypical antipsychotics did not alter CSF glycine or L- and D-serine levels in schizophrenia (Fuchs et al., 2008). Moreover, conventional antipsychotics did not affect immunoreactivity for serine racemase (SRR) or D-amino acid-oxidase (DAO), enzymes involved in the metabolism of glycine and L- and D-serine in the rat brain (Verrall et al., 2007). Taken together, treatment with antipsychotics does not appear to directly affect the levels of the plasma amino acids in question. However, it is important to consider which other clinical features affect the plasma amino acid levels in these schizophrenic patients. The influence of antipsychotics can be excluded in the studies below that enrolled and compared only drug-naive patients with those having schizophrenia and normal controls. Thus, studies enrolling large numbers of drug-naive patients are very important.

3.3. Significance of the degree of changes in endogenous amino acid levels, particularly in peripheral blood

It is important to carefully determine how changes in peripheral blood amino acid levels in patients with schizophrenia (or differences from normal controls) reflect (or influence) amino acid functions in the brain. This question should also be considered in CSF samples for which a magnetic resonance spectroscopy (MRS) study directly measured glutamatergic amino acid levels. We hypothesized based on studies in postmortem brains that schizophrenic pathophysiology is due to chemical changes in schizophrenia. These changes may be much smaller than those observed in other neurological diseases, such as Alzheimer's Disease and Parkinson's Disease, differences that are thought to reflect “minimal (functional) changes” (Ohnuma et al.,