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Symposium title; Biological findings support altered amino acid availability in schizophrenia, bipolar disorders, autism and ADHD

Amino acids and psychiatric disorders, a historical background

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Introduction

Since the 1960ies several routes of research have been followed to find the psychopathological basis of different psychiatric disorders. One of these routes is to study amino acid turnover because tyrosine and tryptophan are the building stones in the synthesis of the monoamines dopamine, noradrenaline, and serotonin (1).

Earlier studies were focused on only a few of the 23 amino acids of the human body. Advanced laboratory techniques, such as high-pressure liquid chromatography (HPLC), have been developed to assess *all* amino acids in blood and CSF simultaneously. Multivariate statistical analyze strategies have been used to reveal possible relationships between amino acid- and monoamine metabolites levels and kinetic variables versus clinical symptoms.

Four factors (A-D) are important to know regarding how amino acid levels influence monoamine turnover. A: The brain is the only organ for which amino acid transport is limited, so that competition for transport occurs at physiological plasma concentrations (2, 3, 4); for a review see (5). B: Tyrosine and tryptophan are essential for the brain, i.e. they cannot be synthesized by the body but must come from the diet. Consequently, the amino acid balance in the periphery influences the transport from plasma to brain and, thereby, on central monoamine metabolism. C: A requisite for normal brain functioning is a continuous availability and balanced amino acid transport from plasma to brain (6). Even small fluctuations of an essential plasma precursor amino acid seem to release psychiatric symptoms including cognitive disturbances (7, 8).

D: Finally, it has also been believed and postulated for many years that the brain can store essential amino acids and in a state of lack of supply from the diet can be compensated by using the amino acids in this storage. However, studies using the positron emission tomography (PET; (9-11) have clearly demonstrated that dietary manipulation of tyrosine by depletion reduces brain dopamine concentration in healthy controls within a few hours. There is *no* storage of amino acids in the brain!

Transport systems for amino acids

In the 1970ies the aromatic amino acids (tyrosine, phenylalanine and tryptophan), were assumed to be transported across membranes, including the blood-brain-barrier, by a common system, the L ("large") system, in competition with other neutral so called branched-chain amino acids (valine, leucine and isoleucine) (3). Another transport system, the A system, was believed at that time only to transport the amino acid alanine ("A" for alanine). However, recently both these systems have been found to have isoforms and transport tyrosine and alanine as well. System L has four isoforms, LAT1-4 (12-15). The LAT1 isoform is the major

transporter of tyrosine coded by a gene on chromosome sixteen (16). This transport system has been shown to compete with alanine for membrane transport (16).

The A system consists of three isoforms, ATA1-3 (17-19). All these transporters are widely expressed in the body and some present in the blood-brain-barrier as well as in fibroblasts (16, 20). The genes coding for them have all been identified in the human genome project and, accordingly, been biochemically described.

There are specific inhibitors for each of these isoforms, which makes it possible to study one or more systems in combination (16, 19). Accordingly, using the fibroblast technique (cf. below) with different inhibitors of amino acid transport it was recently and surprisingly found that 10 percent of tyrosine was transported by the ATA2 isoform (16, 19). Consequently new knowledge on amino acid transport has deeply changed earlier facts about amino acid metabolism and transport and thereby on monoamine metabolism.

Tyrosine, alanine and the first membrane transport

Tyrosine and alanine are essential amino acids (see above) and must be delivered from the food. When studying these amino acids and their end products in different compartments two important facts must be kept in mind:

1. The transport direction goes from the periphery, i.e. from the intestine to plasma (unlimited transport capacity, Figure 1) and then from plasma to the brain (limited transport capacity). Determination of amino acid levels in plasma, CSF and/or the brain with no knowledge of the kinetics of the different amino acid transporters operating in the membranes seems to be of little or no value.
2. The intestine membrane selects amino acids in accordance with existing kinetic conditions i.e. transport velocity and affinity. Indications of a possible transport disturbance can be found in the aminogram and result in closer investigations (21, 22).

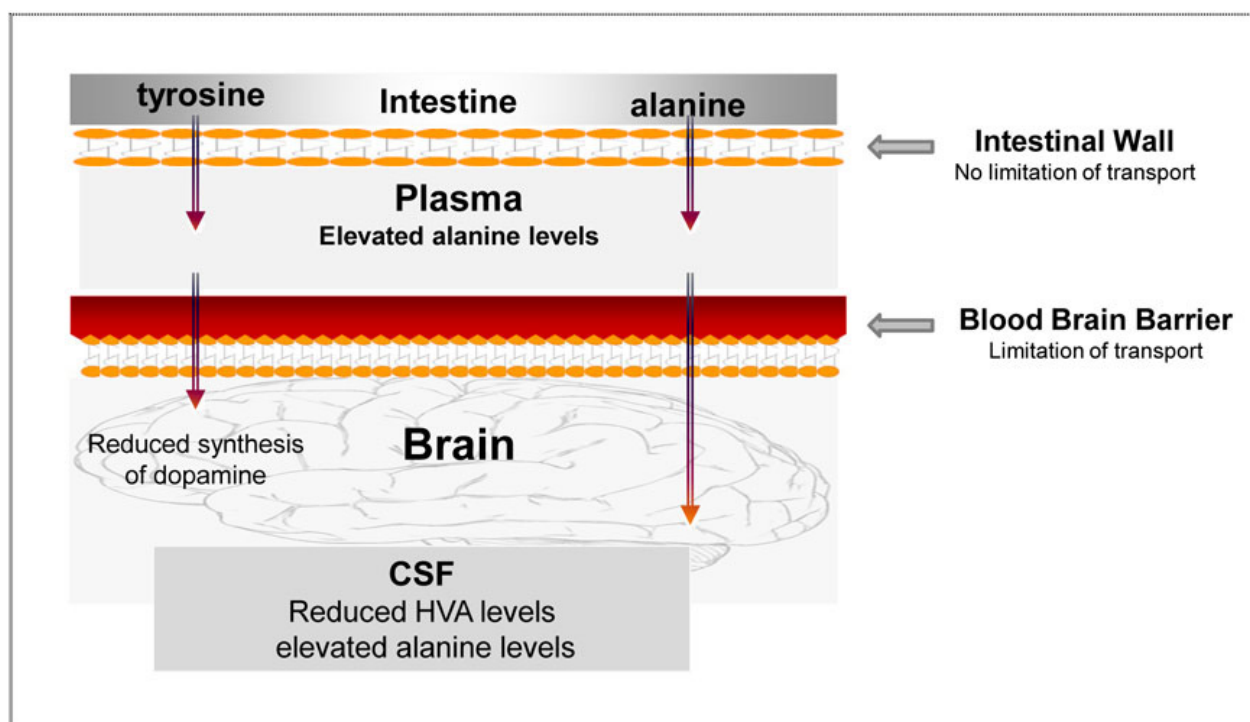


Figure 1.

We have found reduced tyrosine transport in fibroblasts from schizophrenic patients (21, 22), autistic children (23) and bipolar patients (24). In two of these disorders elevated alanine availability expressed as elevated plasma levels in schizophrenic patients (25) and elevated V_{\max} alanine (autistic patients) in fibroblasts was found. The alanine levels compete and reduce the tyrosine transport since they partly use the same transport system (16).

Amino acid transport and schizophrenia

The most consistent finding in schizophrenia research is ventricular enlargement (26) which has been found to be positively correlated with elevated alanine levels in CSF, i.e. the wider ventricles the higher alanine levels. In a study by Revely and coworkers (27) the CSF levels of alanine, glycine, leucine, and phenylalanine were found to be elevated in schizophrenic patients. Thus, three elevated CSF amino acids levels in the latter study (alanine, leucine and phenylalanine) agreed with those that were elevated in plasma (25). Consequently, when studying the turnover and transport of tyrosine, the turnover of alanine must be investigated from the start i.e. plasma to end i.e. CSF. In general CSF levels of amino acids seems to be less reliable since they must be transported over four membranes to reach the brain. Three of them have a limited transport capacity due to competition. Furthermore the levels of amino acids in plasma and CSF are not correlated (25).

PET-Studies on tyrosine transport in schizophrenia

We have performed two investigations to study tyrosine transport from plasma to brain by using the Positron Emission Tomography (PET) technique in schizophrenic patients and healthy controls. In the first study the tyrosine influx over the blood-brain-barrier was found to be lower in patients with schizophrenia than in healthy controls possibly resulting in less tyrosine available in the brain (28). In this PET study, the intransport constant (K_1) did not differ between the patients and the controls. The only explanation for lower influx of tyrosine in the patients was a reduced tyrosine level in plasma. It should be pointed out that there is no limitation of transport between the intestine and plasma (Figure 1). A change in the diurnal rhythm of tyrosine in the schizophrenic patients seems not to be a sufficient explanation since the tyrosine levels are low between 08.00 – 12.00 a.m. (29).

In the second PET study before and after tyrosine loading (Figure 2) it was demonstrated that tyrosine transport was regulated differently in schizophrenic patients compared to controls.

The interpretation of the results in the second PET study was that, since the transport rate (K_1) did not decrease with increasing tyrosine concentrations in patients with schizophrenia, the transport system did not operate at saturation but did so in the controls (30). The reason for this unsaturated transport has up to now not been completely understood. In the first membrane shift, i.e. from the intestine to plasma, significantly higher basal alanine plasma levels were found in the schizophrenic patients as compared to the controls, but no difference in plasma levels of tyrosine (25).

With these facts in mind we suggest that the different regulation of tyrosine transport between schizophrenic patients and controls that was found in the second PET study (30), is caused by competition for transport over the blood-brain-barrier between alanine and tyrosine. The fact that the transport rate (K_1) in the patients did not diminish with increasing tyrosine concentrations, which was the case for the controls, implies that the transport system did not operate at saturation (Figure 3). We suggest that the transporter in the patients was saturated with alanine resulting in increased levels of alanine in the brain and a reduced availability of tyrosine.

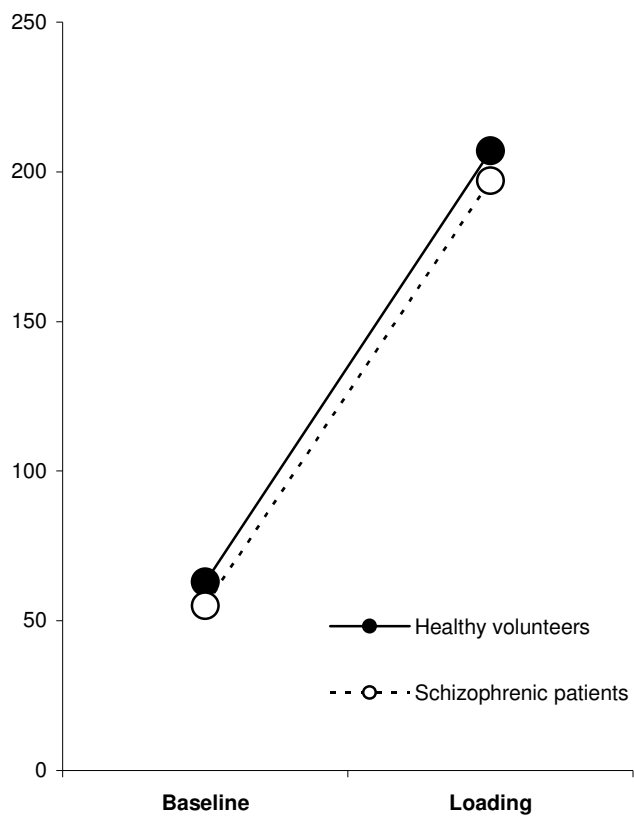


Figure 2.

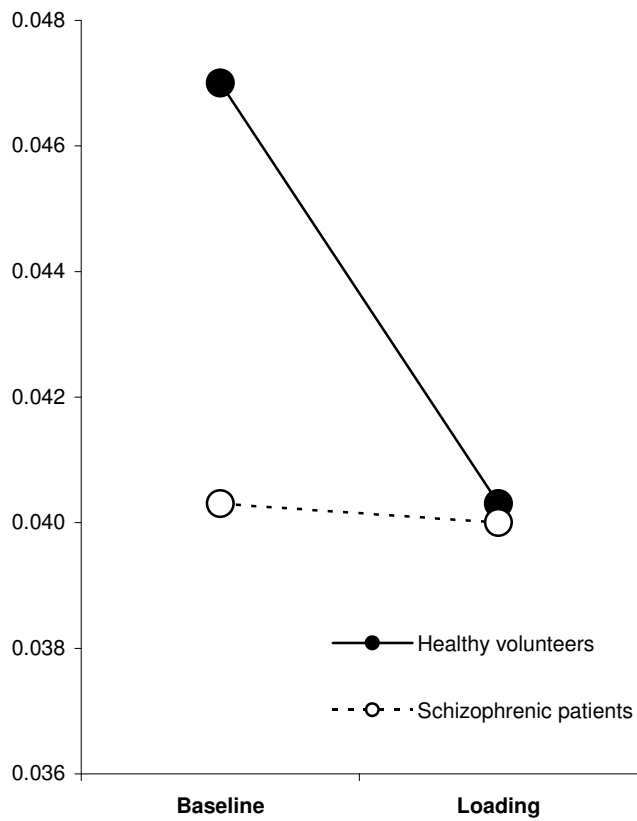


Figure 3.

Cognition and amino acid transport

Deficient tyrosine availability and decreased dopamine synthesis may affect cognition. Neurocognitive dysfunction is frequently observed in schizophrenia, but it has also been reported in other psychotic disorders. This deficit however is most severe and pervasive in schizophrenia (31). Cognitive disturbances have been emphasized in the clinical evaluation of prognosis in schizophrenia (31, 32). Deficiencies in these areas may prevent patients from attaining optimal adaptation and hence act as “neurocognitive rate-limiting factors” and severely affects prognosis.

In a study by Flyckt and co-workers (22) no significant linear relationships were found between the kinetic variables of tyrosine and schizophrenic symptoms. Interestingly, within the same patient group tyrosine transport (K_m) was found to be related to cognitive performance - patients with low K_m had poorer cognitive performance (34) (Figure 4). A similar type of relationship between tyrosine transport and cognition was also found in the healthy mothers of the patients (35). In contrast to Mizrahi et al. (36), who suggested a dissimilar neurobiological substrate for psychiatric symptoms and cognition, we presume a common link between schizophrenic symptoms and disturbed cognition i.e. reduced K_m for tyrosine.

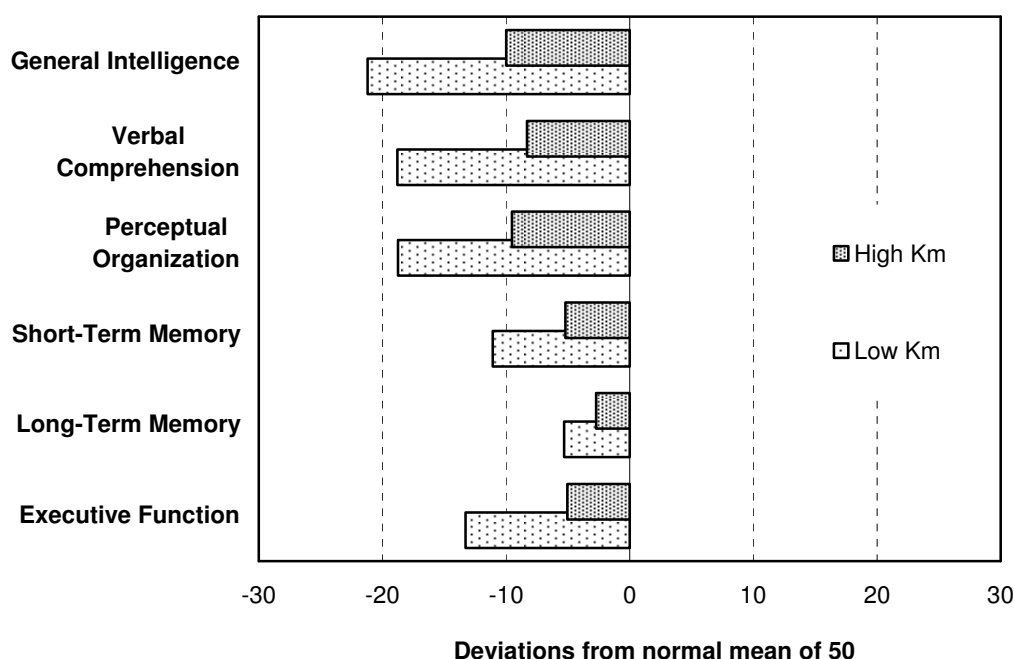


Figure 4.

Genetic findings in amino acid transport

Most interestingly in a large recent study from different centers in the US and Europe (37) a meta-analysis of datasets for multiple psychiatric disorders showed the following: a significant association of an micro duplication of chromosome 16p11.2 with schizophrenia, bipolar disorder and autism. Genes on chromosome 16 seems to be candidate genes for schizophrenia, autism as well as bipolar disorder. It must be underlined that the position of LAT1 is 16q24.3 – accordingly not the same position mentioned above. In the latter three disorders we have found a reduced tyrosine transport resulting in a reduced central dopamine content (21, 22, 23, 24). Rare structural mutations on chromosome 16 of the gene coding for the LAT1 transporter seems likely but other positions on chromosome 16 cannot be excluded.

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Figure legends

Figure 1. Schematic drawing showing the amino acids transport across membranes compartments

Figure 2. Tyrosine plasma levels before (baseline) and after loading in controls and schizophrenic patients

Figure 3. Tyrosine transport in controls and schizophrenic patients

Figure 4. Cognitive functions in schizophrenic patients with low and high K_m