Support for limited brain availability of tyrosine in patients with schizophrenia

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Abstract

Several mechanisms have been suggested to account for altered dopaminergic neurotransmission in schizophrenia. The brain is the only organ for which amino-acid transport is limited and competition for transport over the blood–brain barrier (BBB) occurs at physiological plasma concentrations. One line of research suggests that patients with schizophrenia have altered brain levels of the essential amino acid tyrosine, the precursor for the synthesis of dopamine. The most common hypothesis is that less tyrosine is available because of competition with elevated levels of other amino acids. By consequence, the synthesis of dopamine in the brain will decrease. In contrast, another line of evidence suggests a change in the affinity for one of the transport proteins. A limitation of this research has been that the systems for amino-acid transport across the BBB have not been fully characterized at a molecular or functional level. The L system is the major system for transport of tyrosine across cell membranes including the BBB. The A system is also involved in this transport. Earlier in-vitro studies using fibroblasts have demonstrated a normal L system in schizophrenia but nevertheless reduced tyrosine transport. The combination of molecular research, fibroblast techniques, and brain imaging provides a new basis for clinical research on the role of amino-acid membrane transport in schizophrenia.

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Introduction – the dopamine (DA) hypothesis of schizophrenia

The classical DA hypothesis postulates increased brain dopaminergic transmission (Carlsson, 1978; Haracz, 1982; Seeman et al., 1976; van Rossum, 1966). The hypothesis, which suggests that symptoms arise because of over-activity within the DA system, is mainly based on indirect pharmacological evidence, such as effects of antipsychotic drugs or amphetamine on central dopaminergic neuronal activity.

It has, however, been difficult to obtain consistent evidence for generally increased DA activity in patients with schizophrenia. Although effective for the treatment of positive symptoms hitherto developed, antipsychotics have poor efficacy or may even worsen negative symptoms (Meltzer, 1995). In addition, the psychostimulant D-amphetamine, the precursor D-dopa and the agonist apomorphine may, in some cases, improve negative symptoms (Gerlach and Lühdorf, 1975; Owens et al., 1990; Schaffer et al., 1985; Tamminga et al., 1978; van Kammen and Boronow, 1988). Moreover, a line of neurophysiological observations has provided a theoretical background for a DA dysregulation hypothesis in schizophrenia rather than unidirectional hyperactivity (Svensson et al., 1993; van Kammen et al., 1986).

During recent years, cognitive disturbances have been emphasized in the clinical evaluation of prognosis in schizophrenia (Green, 1996). However, cognitive functions have also been an important area of interest in earlier research on the pathophysiology of schizophrenia. In a study by Weinberger and colleagues it was found that, during the Wisconsin Card Sorting Test, homovanillic acid (HVA) levels in the cerebrospinal fluid (CSF) were positively correlated with...
prefrontal blood flow (Weinberger et al., 1988). The test performance of patients was inferior compared to that of controls. HVA is the major DA metabolite in man and the correlation was interpreted as an indication of decreased cortical dopaminergic activity in schizophrenia. Interestingly two major clinical studies (Bjerkenstedt et al., 1985; Lindström, 1985) and a meta-analysis (Tuckwell and Koziol, 1993) found lower levels of HVA in CSF of neuroleptic-free patients with schizophrenia compared to controls. The assumption that HVA levels in CSF is related to overall neuronal activity has later been supported by the finding of a positive correlation between HVA in CSF and brain glucose metabolism (Wik and Wiesel, 1991). Taken together, the early CSF studies have already provided support for an extension of the original DA hyperactivity theory to a theory based on DA dysregulation.

Positron emission tomography (PET) studies on D1 and D2 DA receptors in neuroleptic-naive patients and stimulant-naive healthy volunteers have demonstrated regional differences, including up- as well as down-regulation of these markers (Abi-Dargham et al., 2003; Farde et al., 1990; Karlsson et al., 2002; Okubo et al., 1997). Strongest support for DA hyperactivity comes from studies showing increased DA release following challenges with amphetamine (Abi-Dargham et al., 2000; Breier, 2001). Because of methodological limitations, the finding has only been reported for the striatum and more sparsely innervated extrastriatal regions have not yet been examined using the PET technique.

Schizophrenia is a devastating disorder that involves a broad spectrum of symptoms (APA, 1994; Feighner et al., 1972; Lieberman, 2001; Wing and Sartorius, 1974). In the classical work of Bleuler (1911), hallucinations and delusions are accessory symptoms while emotional blunting, autism, apathy, and thought disturbances are core symptoms. Later, a dichotomy of positive and negative symptoms was suggested. This dichotomy is supported by pharmacological observations that D2 DA antagonists are effective in decreasing positive symptoms but not effective or may even exacerbate negative symptoms of schizophrenia (Meltzer, 1995). One interpretation of the dichotomy is based on the concepts of state- and trait-dependent symptoms and provides a framework for interpretation of both hyper- and hypodopaminergic neurotransmission in schizophrenia (Svensson et al., 1995). Trait-dependent negative symptoms might be caused by less DA being available for release. State-dependent positive symptoms will appear when excessive DA release is triggered because of disturbed brain function or environmental conditions.

If the DA dysregulation hypothesis of schizophrenia is accepted, the challenge is to mechanistically identify the basis of this disturbance. Accordingly, the primary aim of this article is to review the findings of aberrant tyrosine plasma levels and transport in relation to current research on the DA hypothesis.

**Basal amino-acid physiology**

Tyrosine is an essential amino acid transported from the intestine to plasma and from plasma to the brain. The regulation of amino-acid levels in plasma is not fully understood. Besides dietary factors and diurnal rhythms (Eriksson et al., 1989), the concentrations and metabolism of amino acids are influenced by liver metabolism (Engelhart et al., 2002), protein synthesis (Rebolledo et al., 1974), and several hormones such as insulin (Crandall and Fernstrom, 1983; Freinkel et al., 1968), growth hormone (Simon and George, 1975; Tannenbaum and Martin, 1976), thyroid hormones (Ness et al., 1969), or corticosteroids (Dixit and Buckley, 1967; Feigin et al., 1968; Wurtman et al., 1968). A consequence of the complex regulation of plasma amino-acid levels is that standardized conditions are required in clinical research, comparing patients with schizophrenia and controls.

The blood–brain barrier (BBB) is formed by endothelial cells of brain capillaries and characterized by impermeability of the capillary wall, which is due to the presence of complex tight junctions and low endocytic activity. Amino acids are polar molecules and, thus, cannot pass across cell membranes by passive diffusion. Instead, the amino acids are instead transported across the BBB via facilitated or active transport mechanisms. Importantly, the brain is the only organ for which amino-acid transport is limited and competition between amino acids occurs at physiological plasma concentrations (Pardridge and Oldendorf, 1977).

Tyrosine belongs to the group of large neutral amino acids (LNAA). This group of six amino acids includes tyrosine, tryptophan, and phenylalanine (often called aromatic amino acids), as well as valine, isoleucine and leucine (often referred to as branched-chain amino acids). The six amino acids compete on the sodium-independent L system (L is the abbreviation for ‘large’) for transport across membranes, including the BBB (Pardridge and Oldendorf, 1977). The genes coding for the L system have quite recently been cloned and found to be located on human chromosome 14 (Kanai et al., 1998; Pineda et al., 1999; Prasad et al., 1999; Segawa et al., 1999). There are two isoforms of the L system, L1 and L2. Tyrosine is also
transported from plasma to brain by the sodium-dependent A system (‘A’ is an abbreviation for ‘alanine’) (Hyde et al., 2003; Matthews and Andersen, 2002; Sugawara et al., 2000; Takanaga et al., 2002; Wagner et al., 2001). The genes coding for the A system have recently been cloned and found to be located on human chromosome 12 (Sugawara et al., 2000; Wang et al., 2000). There are two subtypes of the transport protein of the A system – ATA1 and ATA2. The L system is the major transporter of tyrosine, but the A system is also involved. However, the relative importance of the L or the A system for tyrosine transport has up to now not been clarified.

Tyrosine availability

Because of competition at the transport systems, conclusions regarding the effect of amino-acid plasma levels on brain concentrations can only be drawn from studies that include the plasma pattern of all amino acids. Our group has found that untreated patients had different plasma concentrations of 10 amino acids, when compared to controls (Bjerkenstedt et al., 1985). Elevated concentrations were found for alanine, methionine, valine, isoleucine, leucine and phenylalanine, whereas the plasma tyrosine level was within normal range (Figure 1). Moreover, the patients had lower levels of HVA in CSF. Because of the competition for transport, the results suggest that elevated levels of the competing amino acids correspond to less tyrosine in the brain. This interpretation gains support from the finding of negative correlations between HVA in CSF and the elevated plasma amino acids, methionine, valine, isoleucine, leucine and phenylalanine, in the patients with schizophrenia but not in the healthy controls (Bjerkenstedt et al., 1985).

Experimental evidence supports a relationship between low availability of tyrosine and reduced DA concentration in the CNS (McTavish et al., 2001). Indeed, the assumption that plasma levels of tyrosine are related to DA concentration in the brain has received direct experimental support from PET studies in healthy men showing that nutrition with a phenylalanine/tyrosine-free mixture reduces DA release in the human brain (Leyton et al., 2003; Montgomery et al., 2003). In 1986, Leenders and colleagues demonstrated inhibition of L-[18F]dopa uptake into the brain (Leenders et al., 1986).

Fibroblast cell cultures offer a model for experimental studies on the transportation of amino acids across membranes. Using fibroblasts as a model system, we have reported a selective decrease in the transport capacity (V_{max}) of tyrosine into cells from patients with schizophrenia (Hagenfeldt et al., 1987). A limitation of this experimental model is that decreased tyrosine transport could not be attributed to any known amino-acid transport system. The finding was, thus, only interpreted as ‘a more general defect in plasma membrane function in schizophrenia’ (Hagenfeldt et al., 1987; Wiesel et al., 1994). However, with our current knowledge of transport systems it is possible that both L and A systems may be involved in the disturbance of tyrosine transport.

Concerning the A system, it has been found that, when using the fibroblast technique, the addition of alanine decreases tyrosine transport by 50–60% in controls as well as in patients with schizophrenia (Hagenfeldt et al., 1987). This finding supports the hypothesis that the A system may have a physiological role in the transport of tyrosine (Matthews and Andersen, 2002; Sugawara et al., 2000). The low V_{max} for tyrosine may, thus, be linked to the A system, in that the overall capacity (V_{max}) for the amino acids transported by the L system did not differ between fibroblasts from patients and healthy controls (Hagenfeldt et al., 1987).

Low V_{max} for tyrosine transport in fibroblasts from patients with schizophrenia has since been confirmed by Ramchand and colleagues (Ramchand et al., 1996) and Flyckt and colleagues (Flyckt et al., 2001). It has to be emphasized that the calculations of V_{max} have been performed for the total transport across the fibroblast membrane, i.e. the capacity of the A system per se, or has not been investigated. However, such studies can now be performed because the substrate specificity of the A system has been reported and the transport proteins cloned (Sugawara et al., 2000; Wang et al., 2000). The fibroblast studies address attention towards the ATA2 system as this is
Tyrosine and cognitive function

Studies with experimental animals (Tam and Roth, 1997) and human subjects (Deutsch et al., 1994; Diamond, 1994; Griffiths et al., 1995; Shurtleff et al., 1994; Welsh et al., 1990) have shown that variations in brain tyrosine levels may influence brain function. In a study on the effects of methamphetamine, cognitive performance was significantly lower after ingestion of a tyrosine-free mixture compared to a mixture of the DA precursors, tyrosine and phenylalanine (Sharp et al., 1987). Animal experiments support the idea that the functions of the mesoprefrontal DA neurons are influenced by the availability of tyrosine. This part of the DA system has been given particular attention in current research on cognitive deficits in schizophrenia (Goldman-Rakic et al., 2000; Weinberger et al., 1992).

Moreover, the effects of tyrosine on cognitive task performance has been studied in a group of 21 cadets during a demanding military combat training course (Deijen et al., 1999). Ten subjects received five daily doses of a protein-rich drink containing 2 g tyrosine, while 11 subjects received a carbohydrate-rich drink with the same amount of calories (255 kcal). Assessments were made immediately before the combat course and on the sixth day of the course. The group supplied with the tyrosine-rich drink performed better in a memory and a tracking task than the group supplied with the carbohydrate-rich drink. The findings suggest that supplementation with tyrosine reduces the effects of stress and fatigue on cognitive task performance in normal individuals.

The effect of tyrosine on cognition is probably mediated by DA but it cannot be ruled out that increased synthesis of norepinephrine may account for some of the effects. Studies in experimental animals have, however, demonstrated a greater impact of DA than norepinephrine on learning and memory (Myhrer, 2003). More importantly, tyrosine kinetics in patients with schizophrenia has been shown to be connected with cognitive functioning (Wiesel et al., 2005). In conclusion, a series of evidences points to a relationship between availability of tyrosine in the brain and cognitive performance.

Tyrosine in the treatment of schizophrenia

No attempt has been made to treat patients with schizophrenia by tyrosine monotherapy. However, adjuvant therapy has been performed. In a double-blind cross-over study, patients with schizophrenia were treated for 3 wk with either a combination of antipsychotic drugs and 10 g/d tyrosine or antipsychotic drugs and placebo, but no significant improvement was observed (Deutsch et al., 1994). A crucial problem with such a design could be that an ameliorating effect from an elevated level of DA in the brain is inhibited by the global DA antagonistic effect of antipsychotic drug treatment. It should be noted that treatment of patients with Parkinson’s disease with oral L-tyrosine increases DA turnover (Growdon et al., 1982).

In-vivo studies of tyrosine transport

PET is a suitable method to examine tyrosine transport across the BBB. In a PET study tyrosine was labelled with radionucleide carbon-11 (Wiesel et al., 1991). In comparison with healthy controls the tyrosine influx over the BBB was significantly lower in patients with schizophrenia. Moreover, it seemed as if the forward rate constant, $K_1$, in patients was not as sensitive to tyrosine plasma concentrations as in controls. This observation was experimentally addressed in a subsequent PET study, in which two PET measurements of the total transport of $^{11}$C-labelled tyrosine across the BBB were performed on the same day. The first measurement was made during baseline conditions and the second after oral administration of a high dose of L-tyrosine (175 mg/kg) causing markedly elevated plasma levels (Wiesel et al., 1999). Interestingly, the rate constant $K_1$ was reduced by 30% in the controls but virtually unchanged in the patients (Figure 2). The most likely interpretation is that the regulation of transport in patients with schizophrenia is different from that in healthy controls (Wiesel et al., 1999). At a mechanistic level, a plausible interpretation is that the L and A systems for tyrosine transport operate at saturated conditions in the healthy controls but not in the patients (cf. Figure 3). Thus, the PET studies add to the series of observations, in vitro as well as in vivo,
suggesting dysregulated tyrosine transport in patients with schizophrenia.

**Tyrosine and phenylketonuria (PKU)**

The underlying brain dysfunction in phenylketonuria is suggested to be caused by inhibition of tyrosine transport into the brain by competition with the massively elevated plasma levels of phenylalanine (Gardiner, 1990; Pietz et al., 1999). It has also been demonstrated that the administration of high doses of phenylalanine (1000 mg/kg) to rodents inhibits central DA release (During et al., 1988) by competing with tyrosine for transport over the BBB (Fernstrom and Faller, 1978). Indeed, it has been suggested that ‘the transport inhibition may contribute to impaired fetal brain growth in maternal PKU’ (Gardiner, 1990). A more general consequence of disturbed amino-acid transport is that there seems to be an inverse relationship between cerebral protein synthesis and elevated plasma phenylalanine (Pardridge, 1998). The effect of elevated plasma levels of phenylalanine that is due to competition in PKU and the decreased tyrosine transport in schizophrenia may serve as a model for extension of the neurodevelopmental hypothesis of schizophrenia (Weinberger, 1987). In individuals vulnerable to schizophrenia, a reduced tyrosine transport may be particularly crucial during brain growth and maturation.

**Pathophysiological implications**

A dysfunctional tyrosine transport from plasma to brain (cf. above) will affect all the dopaminergic pathways in the brains of patients with schizophrenia, resulting in an aberrant DA metabolism and neurotransmission. Quite recently the ‘synaptic hypothesis of schizophrenia’ was presented (Frankle et al., 2003), proposing a dysfunction of synaptic transmission that results in abnormal connectivity. We suggest that the primary deficit causing abnormal connectivity is a reduced central DA metabolism that is caused by an aberrant tyrosine transport. A change in DA functioning will cause disturbances in other transmitter systems, like the serotonergic and glutamatergic systems.

**Future directions**

It is also known that membrane fluidity may influence signal transmission as well as the enzymic activity in the membrane (Los and Murata, 2000). Membrane fluidity may, thus, influence tyrosine transport by...
a mechanism distinct from the specific transport systems. The fibroblast model may provide an experimental setting for the examination of mechanisms not related to the transport systems because fibroblasts can be incubated with different types of fatty acid – saturated and polyunsaturated. The DA system as well as other transmission systems in the brain may be affected by changes in the regulation of tyrosine transport. While positive symptoms may be controlled by antipsychotic drugs, studies on monotherapy with tyrosine seem of particular interest in patients with residual negative symptoms and cognitive deficits.

Recent data have provided new insight into the molecular biology and functional role of the amino-acid transport systems (Kanai et al., 1998; Pineda et al., 1999; Prasad et al., 1999; Segawa et al., 1999; Sugawara et al., 2000; Wang et al., 2000). A key factor in future research is to determine the functionality and specificity of the L and A systems in the membranes of schizophrenic patients.

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Statement of Interest

None.

References


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