Amino Acid Transport and Receptor Binding Properties in Neuropsychiatric Disorders using the Fibroblast Cell Model

av

Jessica Johansson

Akademisk avhandling

Avhandling för medicine doktorsexamen i medicinsk vetenskap, som enligt beslut av rektor kommer att försvaras offentligt fredagen den 28 oktober 2011 kl. 13.15,
Hörsal P2, Örebro universitet

Opponent: Professor Lars Oreland
Institutionen för Neurovetenskap
Uppsala Universitet

Örebro universitet
Hälsoakademin

701 82 ÖREBRO
Abstract


Altered transport of the catecholamines and serotonin precursor amino acids tyrosine and tryptophan, might be one explanation for the dysfunctional neurotransmission implicated in the pathophysiology of bipolar disorder and Attention Deficit/Hyperactivity Disorder (ADHD). In previous studies, an altered amino acid transport has been found in schizophrenia and autism, when using the fibroblast cell model. The aim of this thesis was to investigate if the transport of precursor amino acids also may be altered in bipolar disorder and ADHD, and to relate the pre-synaptic activity (transport) with post-synaptic activity (receptors). A functional characterization of tryptophan transport in fibroblasts was also motivated, since the transport of tryptophan in fibroblast cells has not been fully explored.

Fibroblast cell lines from patients with bipolar type-1 disorder, from children with ADHD and from controls were included in the studies. The maximal transport capacity ($V_{max}$) and affinity constant ($K_m$) of tyrosine, tryptophan and alanine transport in bipolar patients and ADHD children were determined. Tryptophan transport characterization included; 1) measuring the uptake of tryptophan at high and low concentrations in the presence or absence of transporter selective inhibitors; 2) determination of $V_{max}$ and $K_m$ of tryptophan transport at high and low concentrations; 3) sodium dependency studies of tryptophan uptake. All transport studies were done using the cluster tray method. Furthermore, the maximal binding capacity ($B_{max}$) and the equilibrium dissociation constant ($K_D$) of muscarinic acetylcholine receptors (mAChRs) were determined in the ADHD children by a radioligand binding assay, using the mAChRs antagonist QNB.

In patients with bipolar disorder a decreased $V_{max}$ in the transport of tyrosine was observed ($p=0.027$), while the children with ADHD had a decreased $V_{max}$ of tryptophan transport ($p=0.039$) and an increased $V_{max}$ of alanine transport ($p=0.031$). Children with a hereditary ADHD also had a significantly decreased $B_{max}$ ($p=0.01$). The uptake of tryptophan at both high and low concentrations was partly sodium dependent and the inhibitors had different inhibitory effects on the tryptophan uptake. The uptake of tryptophan at high concentration had low affinity and high $V_{max}$, whilst at low concentration the transport was with high affinity and low $V_{max}$.

Altered amino acid transport was observed in fibroblasts of both bipolar disorder patients and ADHD children, which might indicate that the availability of precursor amino acid in the brain is altered. This could lead to disturbances, directly or indirectly, in the catecholaminergic and serotonergic systems. Children with hereditary ADHD might also have reduced levels of mAChRs in the CNS that could indirectly affect the dopaminergic activity. The uptake of tryptophan was through multiple transporters and was different at different substrate concentrations in terms of sodium dependency, affects of inhibitors and kinetic parameters.

Keywords: Fibroblasts, Bipolar disorder, ADHD, Tyrosine, Tryptophan, Alanine, Transport, mAChRs.

Jessica Johansson, School of Medical Sciences, Örebro University, SE-701 82 Örebro, Sweden, jessica.johansson@oru.se