

N-acetylaspartate in brain - studies on efflux and function

Akademisk avhandling

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Avhandlingen baseras på följande arbeten:

- I. Mattias Tranberg, Malin H. Stridh, Barbro Jilderos, Stephen G. Weber and Mats Sandberg. Reversed-phase HPLC with UV detection for the determination of N-acetylaspartate and creatine.
Analytical Biochemistry (2005) Aug;343(1):179-82
- II. Mattias Tranberg, Malin H. Stridh, Yifat Guy, Barbro Jilderos, Holger Wigström, Stephen G. Weber and Mats Sandberg. NMDA-receptor mediated efflux of N-acetylaspartate: physiological and/or pathological importance?
Neurochemistry International (2004) Dec;45(8):1195-204
- III. Mattias Tranberg, Abdul-Karim Abbas and Mats Sandberg. *In vitro* studies on the efflux of N-acetylaspartate by changed extra- and intracellular osmolarity.
Submitted
- IV. Mattias Tranberg and Mats Sandberg. N-acetylaspartate monomethyl ester increases N-acetylaspartate concentration in cultured rat hippocampal slices: effects on excitotoxicity and levels of amino acids and chloride.
Submitted



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ABSTRACT

N-acetylaspartate (NAA) is an amino acid derivative present in high concentration in the brain. The function of NAA is still unsettled in spite of 50 years of research. The mainly neuronal synthesis and glial breakdown of NAA requires a well regulated neuronal efflux and glial uptake. In the present work hippocampal slices were used to study how NAA efflux from neurons is regulated and to further investigate possible functions of NAA.

For the determination of NAA a reversed phase HPLC method with UV detection was developed. The method allowed for the simultaneous determination of NAA and creatine and was comparable or better in sensitivity than previous methods based on UV detection.

A newly developed efflux protocol that allowed the determination of efflux and delayed cell death was used to study NAA efflux in cultured hippocampal slices. Activation of the NMDA receptor, a glutamate-receptor subtype that is involved in learning and memory but also in nerve cell death following stroke, evoked a prolonged Ca^{2+} -dependent NAA efflux from cultured slices. The efflux of NAA was not due to unselective membrane rupture but at high NMDA concentrations the efflux of NAA correlated with the NMDA-mediated *delayed* (24 hours after efflux) excitotoxicity. However, no causal relationship between delayed excitotoxicity and extracellular NAA could be demonstrated as culturing with high concentrations of NAA was non-toxic.

Extracellular osmolarity was decreased moderately for 10-48 hours to address the proposed function of NAA as an osmoregulator but no change in the tissue content of NAA was observed from either cultured or acutely prepared hippocampal slices. However, depolarisation resulted in efflux of NAA from acutely prepared slices that could be reduced both by a NMDA-receptor blocker and hyperosmotic solution.

Culturing of hippocampal slices with the monomethyl ester of NAA increased intracellular NAA levels. This was followed by reduced levels of the anion phosphoethanolamine and a tendency towards decreased Cl^- concentration in the slices. NMDA-mediated delayed excitotoxicity was unaffected by increased intracellular NAA concentration.

Overall, the results suggest that the NMDA receptor is involved in the regulation of NAA efflux from neurons. Increased extracellular as well as intracellular NAA is non-toxic and NAA does not seem to function as an important Ca^{2+} chelator or as an osmoregulator under physiological decreases in osmolarity.

Keywords: N-acetylaspartate, hippocampal slice cultures, HPLC, NMDA, excitotoxicity, volume regulation, Canavan disease

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