GLIAL HEMICHANNELS:
A NEW ROUTE FOR CHEMICAL COMMUNICATION IN BRAIN

Akademisk avhandling

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The thesis is based on the following papers:

I. 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

II: Malin H Stridh, Mattias Tranberg, Stephen G. Weber, Fredrik Blomstrand and Mats Sandberg
Stimulated efflux of amino acids and glutathione from cultured hippocampal slices by omission of extracellular calcium: likely involvement of connexin hemichannels
Journal of Biological Chemistry (2008) Feb; doi:10.1074/jbc.M704153200

III: Malin H Stridh, Stephen G. Weber, Fredrik Blomstrand, Michael Nilsson and Mats Sandberg
Stimulated efflux of adenosine via astroglial connexin hemichannels
Submitted to Neuroscience letters

IV. Malin H Stridh, Stephen G. Weber, Fredrik Blomstrand, Michael Nilsson and Mats Sandberg
Characterization of glutathione efflux from astroglial connexin hemichannels
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Abstract:

The extracellular neurochemistry determines normal brain function and the faith of neurons after insults such as stroke. This thesis concerns the effect of extracellular events related to intense neuronal stimulation and stroke, i.e. over-activation of glutamate-receptors and dramatically decreased extracellular Ca^{2+}-concentrations, on efflux of neurotoxic and neuroprotective substances. The use of cultured slices of rat hippocampus enabled parallel analysis of efflux in combination with determination of delayed nerve cell death after brief (5 min) overactivation of NMDA-receptors or omission of extracellular Ca^{2+} for 15 min. Efflux by NMDA-receptor stimulation was selective and dominated by N-acetylaspartate, the antioxidant glutathione, phosphoethanolamine, taurine and hypotaurine. The efflux induced by concentration at and above 60 μM NMDA was paralleled by delayed neurotoxicity 24 h later. The efflux pathway is still unknown but does not appear to involve hemichannels, the Ca^{2+}-calmodulin dependent kinase II or NO-synthesis. Efflux activated by omission of extracellular Ca^{2+} for 15 min caused an efflux pattern from cultured slices that was dominated by glutathione but lacked N-acetylaspartate, indicating efflux originating from glial cells. This efflux was blocked by gap junction blockers, carbenoxolone, flufenamic acid and endothelin-1, which indicated efflux from activated so called hemichannels (half gap junctions). The involvement of hemichannels was further strengthened by the inhibitory effect of a mimetic/blocking peptide for Cx43, the major connexin-protein in astroglial cells. Inhibitors of other putative channels, the $P2X_7$-receptor and pannexin hemichannels, were without effect. Volume regulated channels were probably not involved as hypertonic medium did not reduce the efflux stimulated by omission of extracellular Ca^{2+}. The efflux was mainly of glial origin as cultured slices in which neurons had been degenerated showed similar efflux pattern by omission of Ca^{2+}. These results together showed that omission of extracellular Ca^{2+} activate opening of glial connexin hemichannels. Omission of extracellular Ca^{2+} did not induce delayed nerve cell death as long as glutamate uptake was intact. However, using glutamate uptake blockers revealed that opening of glial hemichannels resulted in glutamate efflux which caused delayed neurotoxicity and efflux of N-acetylaspartate, i.e. effects similar to that induced by NMDA-receptor overactivation. In another set of experiments the efflux induced by Ca^{2+}-omission from primary astroglial cultures was characterized. Using inhibitors for $P2X_7$-receptors, gap junctions and connexin hemichannels demonstrated efflux of the neuroprotective substance adenosine via connexin hemichannels. It was also shown that curcumin, an agent which activate a transcription factor which in turn induce transcription of a multi-fold of antioxidant genes, dramatically increase both efflux and intracellular levels of glutathione. The main finding of the work is that opening of astroglial connexin hemichannel cause efflux of neuroprotective substances. However, opening of hemichannels in conditions with reduced capacity for glutamate uptake, such as stroke, can cause additional neurotoxicity.

Keywords: astrocyte, antioxidant, connexin, hemichannel, glutathione, efflux, glutamate

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