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Title: Mitochondrial localization of two brain proteins, $p42^{IP4}$/centaurin-α1/ADAP1 and CNP, and their involvement in regulation of mitochondrial Ca^{2+}

Abstract

The neuronal protein $p42^{IP4}$ was suggested to be involved in neurodegenerative processes. To find out a role of $p42^{IP4}$ in cell death, we detected apoptosis in control and $p42^{IP4}$ overexpressing mouse neuroblastoma (N2a) cells by caspase-3 assay, DNA-laddering assay and flow cytometry analysis. We have shown that $p42^{IP4}$ is not involved in apoptosis. However, we observed that $p42^{IP4}$ had an effect on cell cycle.

Cellular Ca^{2+} signals are crucial in the control of most physiological processes, cell injury and cell death. Mitochondria play a central role in cellular Ca^{2+} signalling. During cellular Ca^{2+} overload, mitochondria take up cytosolic Ca^{2+}, which, in turn, can lead to opening of the permeability transition pore (PTP). Although Ca^{2+}-dependent PTP has been implicated in a broad range of cell death pathways, the exact mechanism of the PTP opening remains elusive.

Previously, $p42^{IP4}$ was identified in brain membrane fraction, which also contained mitochondria. Some other data indicate possible localization of $p42^{IP4}$ in mitochondria. Therefore, this important question was studied here.

We determined for the first time mitochondrial localization of $p42^{IP4}$. Moreover, in rat brain mitochondria (RBM), we found interaction of $p42^{IP4}$ with 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNP) and α-tubulin by pull-down binding assay and by immunoprecipitation.

Localization of $p42^{IP4}$ and CNP in the inner membrane fraction of mitochondria prompted us to study whether $p42^{IP4}$ and CNP are involved in regulation of mitochondrial Ca^{2+}-induced PTP. Simultaneous measurements of the respiratory rate, trans-membrane potential, and Ca^{2+} transport in the mitochondrial suspension were performed. We determined the rate of Ca^{2+} influx, Ca^{2+} capacity and lag time for PTP opening in mitochondria isolated from $p42^{IP4}$-transfected and from control N2a cells. Overexpression of $p42^{IP4}$ led to promotion of Ca^{2+}-induced PTP opening. Furthermore, $p42^{IP4}$ ligands, phosphatidylinositol(3,4,5)trisphosphate and inositol(1,3,4,5)tetrakisphosphate, accelerated PTP opening in mitochondria isolated from N2a cells.

We found the interaction of CNP with modulators of PTP, adenine nucleotide transporter and voltage-dependent anion channel. The enzymatic activity of CNP was reduced under PTP opening. Involvement of CNP in PTP operation was confirmed in experiments using mitochondria isolated from CNP-knock-down oligodendrocyte cell line (OLN93). In mitochondria isolated from OLN93 cells transfected with CNP-targeting small interfering RNA, CNP reduction was correlated with facilitation of Ca^{2+}-induced PTP opening. The CNP substrates, 2',3'- cyclic AMP and 2',3'- cyclic NADP, enhanced PTP development in RBM.

In summary, our results suggest that $p42^{IP4}$ and CNP in mitochondria play a role in regulation of mitochondrial Ca^{2+} transport mechanisms in the brain. While Ca^{2+}-induced PTP opening is important stage of initiation of cell death, consequently we hypothesize that in the brain $p42^{IP4}$ and CNP contribute to processes leading to neurodegenerative diseases.