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BIOLOGICAL ROLE OF NICOTINIC ACETYLCHOLINE RECEPTORS IN MITOCHONDRIA

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Introduction. Nicotinic acetylcholine receptors (nAChRs) are classically regarded as ligand-gated ion channels located in the cell plasma membrane to mediate fast synaptic transmission in muscles and neurons. In addition, they are present in many non-excitable cells to regulate cell survival, proliferation, adhesion and production of cytokines. We have found that α7β2, α3β4, α4β4 nAChR subtypes are located in the outer membrane of mitochondria to regulate the early stage of mitochondrial apoptosis, namely, the opening of mitochondrial pore, through which cytochrome *c* (cyt *c*) is released under the effect of apoptogenic agents like Ca^{2+} or H_2O_2. Cyt *c* release from mitochondria can be attenuated by either nAChRs agonist or antagonist indicating that the nAChR signaling in these intracellular organelles is ion channel-independent and is mediated through conformational changes of the nAChR molecule upon specific ligand binding. The lack of α7, α3 or α7 and β2 nAChR subunits in mitochondria of mutant (knockout) mice was compensated by significant increase of α9 and β4 subunits. Correspondingly, mutant mice mitochondria did not change dramatically their response to Ca^{2+}, but were affected by subtype-specific ligands in different way compared to mitochondria of the wild-type mice. The aim of the present study was to evaluate the role of nAChRs in mitochondria under different physiological conditions.

Methods. Experiments have been performed in C57Bl/6 mice and Wistar rats. Mitochondrial and non-mitochondrial fractions were isolated from the liver, brain, lung or lung carcinoma by differential centrifugation according to standard procedures. The level of nAChR subunits was studied by Sandwich-ELISA using subtype-specific antibodies. The apoptotic resistance of live mitochondria was evaluated based on the level of cyt *c* released under the effect of Ca^{2+} and H_2O_2.

Results. It was found that neuroinflammation caused by either LPS injection or immunization with α7(1-208) resulted in decreased level of α7 nAChRs in the mouse brain mitochondria compared to mitochondria of control mice. In functional assay, mitochondria of LPS-treated or α7(1-208)-immunized mice released more cyt *c* in response to Ca^{2+} and were less sensitive to attenuating effect of α7-specific agonist PNU282987 than mitochondria of control mice. Treating mice with N-stearoylethanolamine (NSE) prevented α7 nAChR decrease in the brain mitochondria making them more resistant to apoptogenic effect of Ca^{2+}. In contrast, increased level of a7 nAChRs was observed in mitochondria purified from Lewis lung carcinoma compared to normal mouse lung, making mitochondria less sensitive to low doses of Ca^{2+}. Finally, the increase of a7-, a3-, α4- and, especially, a9-containing nAChRs was found in the rat liver mitochondria 3-6h after partial hepatectomy resulting in increased mitochondria resistance to 0.1-0.9 μM Ca^{2+} and 0.1-0.5 mM H_2O_2.

Conclusions. These data demonstrate a physiological significance of mitochondrial nAChRs in supporting mitochondria sustainability to apoptogenic influence. The level of α7 nAChRs is critically important upon neuroinflammation, while up-regulation of α9- and β4-containing nAChRs in mitochondria is a physiological response to either compensate the deficiency of other nAChR subtypes or support the cell survival in critical circumstances.

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