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Characterization of the human D-amino acid oxidase (hDAAO) - pLG72 complex involved in the onset of schizophrenia

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Schizophrenia is a chronic and severely debilitating psychiatric disorder affecting nearly 1% of the world's population. In 2002, the new human gene G72, encoding for the pLG72 protein, and the gene encoding for D-amino acid oxidase (DAAO) have been genetically linked to the susceptibility to schizophrenia. A yeast two-hybrid screening experiment identified DAAO as a putative interacting partner of pLG72. DAAO is a FAD-containing flavooxidase that in brain is responsible for the elimination of D-serine, a co-agonist that binds to the glycine-site of the NMDA receptor. We recently demonstrated that pLG72 acts as "inactivator" of human DAAO (hDAAO) and that the cellular concentration of D-serine might depend on the expression of the active form of this flavooxidase. Based on these results, a molecular model for the onset of schizophrenia has been proposed: a decrease in pLG72 expression might yield an anomalous high level of hDAAO activity and therefore a decrease in the local concentration of D-serine, affecting glutamatergic neurotransmission mediated by NMDA receptor.
The characterization of the complex is a challenging task, hardly feasible by high resolution techniques. The main limit is due to pLG72 since: 1) no structural information is available; 2) it is soluble only in the presence of mild denaturant; 3) no homologous proteins have been characterized so far. In this perspective, we have used low resolution strategies based on the coupling of classical biochemistry approaches (complementary proteolysis, cross-link) with mass spectrometric techniques, to characterize the pLG72-hDAAO complex. Results indicated that hDAAO exhibits different proteolysis profiles when isolated or in complex with pLG72, thus suggesting a conformational change upon binding the effector protein. Chemical cross-linking experiments will complement the proteolysis experiments providing with details about the contact regions between hDAAO e pLG72.

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Brain mitochondria play a major role in cellular hypoxia sensing

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Hypoxia leads to massive release of neurotransmitter glutamate during ischemic stroke. Elevation of concentration of intracellular calcium via NMDA receptor under glutamate excitotoxicity results in inhibition of mitochondria function including permeability of mitochondrial transition pore, $\Delta\psi$ loss and release of proapoptotic factors. Meanwhile, it is possible to assume direct signaling pathway linked hypoxia and mitochondria. Our aim was to study the mitochondria hypoxia sensing mechanism.
We used three models: rat brain homogenates after *in vivo* common carotid artery occlusion, homogenates of cortex tissue slices, and mitochondria isolated from brain using Percoll density centrifugation. Using *in vivo* model we have shown that after 24 hours of occlusion in rats respiratory control index (RCI) of mitochondria was reduced from 7.7 ± 0.5 to 4.5 ± 0.3. The same disruption of phosphorylating system was observed in both *in vitro* models of hypoxia (brain slices and isolated brain mitochondria). The reduction of RCI was accompanied with increased of H_2O_2 generation by isolated mitochondria. The effects of a modulator (glycine) and an inhibitor (MK-801) of NMDAR on mitochondrial function were studied. The depletion of RCI in both *in vivo* and *in vitro* models was effectively prevented by glycine. Generation of H_2O_2 by mitochondria stimulated in anoxia was also significantly reduced by MK-801 (10 μM) and glycine (5 mM).
We suggest that one of the possible mechanism of hypoxia sensing by mitochondria could be associated with abundance of the functional NMDA receptor in the brain mitochondria. NMDAR agonist glutamate (10–500 μM) added to isolated mitochondria in the presents of rotenon-e (inhibitor of complex I mitochondrial respiratory chain) stimulates the generation of H_2O_2 up to three-folds. Process was significantly reduced by 10 μM MK-801 (inhibitor NMDAR) and 5 mM glycine.
These findings suggest a novel mechanism by which brain mitochondria could directly sense hypoxia during stroke.

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P2X7 antagonists improve "*in vitro*" myelination in organotypic dorsal root ganglia (DRG) cultures from a rat model of CMT1A neuropathy.

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Charcot-Marie-Tooth 1A (CMT1A) is a hereditary neuropathy associated with overexpression of the peripheral myelin protein 22 (PMP22), causing demyelination. The molecular mechanisms leading to Schwann cell (SC) dysfunction are not understood and no treatment is available for CMT1A.
We reported an abnormally high intracellular Ca^{2+} concentration (Ca^{2+}) (i) in SC from a rat model of CMT1A (CMT1A SC), caused by overexpression of the purinergic receptor P2X7. Correction of the elevated (Ca^{2+}) (i) levels by the use of P2X7 antagonists or through down-regulation of P2X7 expression restored the normal phenotype in CMT1A SC.
We recently identified a new P2X7 antagonist, called P18, which is an isomer of Ap2A. P18 is also an agonist of P2Y11, the only purinergic receptor increasing the (cAMP) (i) a positive regulator of SC differentiation.
Organotypic DRG cultures from both wt and CMT1A rats were treated for 3 weeks with 200 nM P18, or 1 μM A438079 (a commercially available P2X7 antagonist), or 1 mU/ml apyrase. All treatments significantly increase expression levels of the myelin protein MPZ (by 1.3-, 2.1- and 1.5-fold in CMT1A SC, by 1.4-, 1.1- and 2.3- in wt SC, respectively, as determined by western blots). Morphometric analysis of DRG, treated with P18 or A438079, and stained with Sudan black, confirms a significant increase of myelin segment density compared to untreated DRG. Neurofilament dephosphorylation levels, a measure of the possible detrimental effect on neurons, are not increased by the treatments.