Effect of *in vivo* administration of L-acetylcarnitine on ATP-ases of synaptic plasma membranes of rat striatum

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Synaptic malfunction and degeneration have been shown to contribute to pre-clinical and early-symptomatic stages of many neurodegenerative conditions, like Alzheimer's Disease and Parkinson's Disease (PD). Maintenance of synaptic structure and functionality is a highly energy-dependent process (MacAskill and Kittler, 2010, Villa et al., 2012). Thus, studying brain bioenergetics at synaptic level is an useful approach to evaluate new potential therapeutic molecular targets for these pathologies, as shown in studies evaluating the effect of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) administration on monkeys brain metabolism (Villa et al., 1992, 1994). L-acetylcarnitine (LAC) is a naturally occurring derivative of carnitine (3-hydroxy-4-N-trinethylaminobutyric acid), influencing synaptic transmission through the increase of mitochondrial oxygen utilization (Villa and Gorini, 1991; Gorini et al., 1998) and of cardiolipin content in the inner mitochondrial membrane (Hagen et al., 1998), stabilizing the membrane itself.

Thus, the aim of this study on *functional proteomics* was to evaluate the effect of LAC subchronic *in vivo* treatment at two different doses (30 and 60 mg x kg$^{-1}$ i.p., 28 days, 5 days/week) on the maximum rate ($V_{\text{max}}$) of the following representative enzymes linked to energy-utilizing systems (ATP-ases): (i) Na$^+$, K$^+$-ATP-ase; (ii) Na$^+$, K$^+$, Mg$^{2+}$-ATP-ase; (iii) ouabain insensitive Mg$^{2+}$-ATP-ase; (iv) Ca$^{2+}$, Mg$^{2+}$-ATP-ase; (v) High- and (vi) Low-affinity Ca$^{2+}$-ATP-ases; (vii) the ectoenzyme Mg$^{2+}$-ATP-ase. Also (viii) acetylcholinesterase (AchE) was assayed. These enzyme activities were evaluated on their *in vivo* localization *i.e.* on synaptic plasma membranes (SPMs, two types: SPM1 and SPM2), obtained from striatum of single rats (4 month-old female Sprague-Dawley), so to consider the brain structural, functional and metabolic macro-heterogeneity of a selectively vulnerable area to PD.

The results showed that in control (vehicle-treated) animals, enzymatic activities are differently expressed in SPM1 respect to SPM2, being higher in SPM2. LAC treatment exerted its effects differently in relation to the type of SPMs: (i) AChE decreased on SPM1 and SPM2 at the dose of 30 mg x kg$^{-1}$; (ii) ouabain insensitive Mg$^{2+}$-ATP-ase and High-affinity Ca$^{2+}$-ATP-ase activities decreased on SPM1 respectively at the dose of 30 and 60 mg x kg$^{-1}$; (iii) Na$^+$, K$^+$-ATP-ase, Ca$^{2+}$, Mg$^{2+}$-ATP-ase and Mg$^{2+}$-ATP-ase activities decreased at the dose of 30 mg x kg$^{-1}$ on SPM2; (iv) Low-affinity Ca$^{2+}$-ATP-ase activity was unaffected.

These data show that LAC decreased the striatal energy consumption linked to modulation of resting transmembrane potential and intracellular ions and Ca$^{2+}$ homeostasis, interfering also with cholinergic neurotransmission. These modifications are qualitatively similar to those observed during Parkinson's-like syndrome induced by MPTP (Calabresi et al., 2006). Comparing these results with those obtained in rat cerebral cortex (Villa et al., 2011), LAC has a specific effect on enzyme activities according to the considered cerebral area. This confirms that cellular subfractionation is an useful method to study the pharmacodynamic characteristics of drugs acting on cerebral energy metabolism, taking into account the macro-heterogeneity of cerebral areas (*i.e.* striatum) and micro-heterogeneity of brain ATP-ases systems (SPM1 and SPM2), whose individual roles are determined by their specific subcellular *in vivo* localization.


