Bioenergetic effects of Clonidine on functional proteomics

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Clonidine is an anti-hypertensive drug that binds to pre-synaptic alpha$_2$-adrenoreceptors and inhibits the release of extra-cellular norepinephrine. Being liposoluble, Clonidine crosses the blood-brain barrier producing many different central pharmacological effects. So, this drug is investigated also for its possible cerebroprotective effects towards hypoxia-ischemia. In fact, because the stimulation of the sympathetic nervous system increases the metabolic rate in various tissues, some studies from heterogeneous experimental settings (*i.e*. pre-operative procedures, endotoxemia, severe head trauma) seem to suggest that this drug may be able to decrease energy expenditure (Schmidt et al., 2013).
For these considerations and because of the lack of data on the functional proteomic parameters regulating brain energy metabolism, in this study, the effects of acute Clonidine treatment (5 microg x kg^{-1}, 30 minutes) on the maximal rate (V_{max}) of representative and regulatory mitochondrial enzyme activities related to Krebs' cycle, electron transport chain and glutamate and related amino acids metabolism were systematically assessed as a measure of tissue energy transduction potentialities (Ferrari et al., 2015; Villa et al., 2013a).
Because of the heterogeneity of brain mitochondria (Villa et al., 2012a, 2012b, 2013b), this research was performed (a) on non-synaptic mitochondria, *i.e*. the mitochondrial population located *in vivo* in neuronal perikaryon, and (b) on intra-synaptic mitochondria (two populations, light and heavy) located *in vivo* in synapses. These types of mitochondria were purified from the cerebral cortex of male Sprague-Dawley rats aged 3 months.
In control animals, enzyme activities varied in a complex way respect to the type of mitochondria. This micro-heterogeneity is an important factor, because energy-related mitochondrial enzyme catalytic properties cause metabolic modifications of physiopathological significance in the cerebral tissue *in vivo*, discriminating pre- and post-synaptic sites of drug actions and affecting tissue responsiveness to noxious stimuli (Moretti et al., 2015; Villa and Gorini, 1997).
Clonidine acute treatment modified some enzyme activities in all the tested mitochondrial populations: (i) in non-synaptic mitochondria, citrate synthase, cytochrome oxidase and glutamate-oxaloacetate transaminase activities were increased by the drug; (ii) in intra-synaptic light mitochondria, citrate synthase was decreased and cytochrome oxidase and glutamate-oxaloacetate transaminase activities were enhanced; (iii) in intra-synaptic heavy mitochondria, citrate synthase, cytochrome oxidase and glutamate dehydrogenase activities were reduced.
The different effects of Clonidine on the same enzymatic activities when evaluated on mitochondria from specific intracellular sites may represent the translation to the biochemical machinery of the chemical message of the drug, that exerts a diversified effect on pre- and post-synaptic compartments.
In fact, Clonidine acute treatment increased the neuronal energy state of the cerebral cortex particularly in post-synaptic compartment. These results related to the effects of Clonidine on brain energy metabolism are useful to better understand the molecular mechanism of action of this drug. Thus, this functional proteomic approach represents an innovative pharmacological strategy to evaluate (i) the diversified effects of Clonidine on brain mitochondria, (ii) mitochondrial dysfunctions and (iii) how drugs are able (or not) to interfere *in vivo* with the mitochondrial energy transduction potentialities.

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