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EFFECT OF DESIPRAMINE AND FLUOXETINE ON ENERGY METABOLISM OF CEREBRAL MITOCHONDRIA

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Abstract—Brain bioenergetic abnormalities in mood disorders were detected by neuroimaging *in vivo* studies in humans. Because of the increasing importance of mitochondrial pathogenetic hypothesis of Depression, in this study the effects of sub-chronic treatment (21 days) with desipramine (15 mg/kg) and fluoxetine (10 mg/kg) were evaluated on brain energy metabolism. On mitochondria *in vivo* located in neuronal soma (somatic) and on mitochondria of synapses (synaptic), the catalytic activities of regulatory enzymes of mitochondrial energy-yielding metabolic pathways were assayed. Antidepressants *in vivo* treatment modified the activities of selected enzymes of different mitochondria, leading to metabolic modifications in the energy metabolism of brain cortex: (a) the enhancement of cytochrome oxidase activity on somatic mitochondria; (b) the decrease of malate, succinate dehydrogenase and glutamate-pyruvate transaminase activities of synaptic mitochondria; (c) the selective effect of fluoxetine on enzymes related to glutamate metabolism. These results overcome the conflicting data so far obtained with antidepressants on brain energy metabolism, because the enzymatic analyses were made on mitochondria with diversified neuronal *in vivo* localization, *i.e.* on somatic and synaptic. This research is the first investigation on the pharmacodynamics of antidepressants studied at subcellular level, in the perspective of (i) assessing the role of energy metabolism of cerebral mitochondria in animal models of mood disorders, and (ii) highlighting new therapeutical strategies for antidepressants targeting brain bioenergetics.

Key words: desipramine, fluoxetine, mitochondria, Functional Proteomics, brain energy metabolism.

INTRODUCTION

Although the great variety of antidepressant drugs (ADs), therapy of Depression presents several open issues, particularly the delayed therapeutical response, *i.e.* 2–3 weeks from the start of treatment, and the different mechanisms of action of various classes of ADs; in addition, only 60–70% of treated patients successfully respond to therapy.

Acute AD treatment increases norepinephrine (NE) and 5-hydroxytryptamine (5-HT) concentrations, triggering the subsequent pharmacological events leading to the therapeutical effects after subchronic and chronic treatments, probably related to adaptive responses to ADs of the central nervous system (CNS) (Hyman and Nestler, 1996).

Therefore, the "biogenic amine hypothesis" should be revised to consider receptor plasticity, *i.e.* the downregulation (reduced number) and desensitization (diminished responsiveness) of pre- and post-synaptic receptors (Moretti et al., 2003). Receptor downregula tions/desensitizations are indicative of sustained receptor activation, secondary to continued increases of monoamine levels after subchronic and chronic AD treatments.

Long-term treatments result in prolonged activation of intracellular signal transduction that mediates the stimulation of monoamine receptors (Hamon and Blier, 2013). In fact, a large body of evidence (Manji, 1992; Racagni et al., 1992; Warsh and Li, 1996; Perez et al., 2000; Brunello and Tascedda, 2003) indicates that: (i) changes of the transduction pathways of NE or 5-HT receptors are associated with affective disorders and (ii) AD and Lithium treatments entail adaptive responses due to the activation of these pathways.

All these processes closely depend on energy metabolism, being ATP availability needed for G-protein activity, cAMP formation, phosphatidylinositol moieties, protein kinase activities, transport of ions, neurotransmitters re-uptake and protein synthesis (Moretti et al., 2003).

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Abbreviations: 5-HT, 5-hydroxytryptamine; ADs, antidepressant drugs; CCRS, NADH-cytochrome *c* Reductase Rotenone-sensitive; CCRT, NADH-cytochrome *c* Reductase; CNS, central nervous system; COX, cytochrome oxidase; CS, citrate synthase; ETC, Electron Transport Chain; FM, Somatic "Free" Mitochondria; GLDH, glutamate dehydrogenase; GOT, glutamate-oxaloacetate transaminase; GPT, glutamate-pyruvate transaminase; HM, Synaptic "Heavy" Mitochondria; LM, Synaptic "Light" Mitochondria; MDH, malate dehydrogenase; NE, norepinephrine; SDH, succinate dehydrogenase.

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Interestingly, brain energy metabolism abnormalities in mood disorders were detected also by neuroimaging *in vivo* studies in human patients. Firstly, the observations were made on Cerebral Blood Flow and Cerebral Metabolic Rate of glucose (Drevets, 1999, 2000; Stoll et al., 2000), increasing thereafter the depth of analysis evaluating the modifications of energy-linked metabolites and molecules (Pettegrew et al., 2002; Deicken et al., 2003; Iosifescu and Renshaw, 2003; Price and Drevets, 2012).

However, in contrast with the neuroimaging findings indicating the restoration of brain bioenergetics after AD treatment, some experimental studies suggest that ADs are inhibitors (Byczkowski and Borysewicz, 1979; Weinbach et al., 1986; Souza et al., 1994), while for others ADs are stimulants of mitochondrial energy metabolism (Katyare and Rajan, 1995; Zhang et al., 2008). Thus, a *consensus* on this issue has not been reached yet.

These conflicting results may be due to a variety of factors, including: (i) biases in experimental study design, *e.g.* using whole brain homogenates and the bulk of cerebral mitochondria; (ii) not taking into account the heterogeneity of brain areas as regards the bioenergetic changes observed in depressed patients (Drevets, 2000); (iii) the lack of *in vivo* analyses of energy abnormalities at subcellular level in Depression and AD therapy, not considering a possible antidepressant differential effects on pre-synaptic nerve endings and on post-synaptic terminals, as we have done.

Thus, the present study evaluated the effects of 21-day treatment with desipramine and fluoxetine on brain energy metabolism of the rat frontal cortex, through the assay of the activity of regulatory enzymes (*Functional Proteomics* or *Functional Enzymology*) of mitochondrial energy-yielding metabolic pathways. This research was performed on: (a) somatic mitochondria (FM) and (b) synaptic mitochondria [“light” (LM) and “heavy” (HM)] located *in vivo* in synapses (Villa et al., 1989, 2013a).

Due to their localization, these mitochondria are different on their bioenergetic basis because the Gibbs free energy ($\Delta G^{0'}$) of somatic mitochondria, related to the post-synaptic neuronal compartment, is linked to protein assembly; otherwise, that of synaptic ones are linked to ions homeostasis and neurotransmission.

Overall, this functional approach allows to study the metabolic changes occurring in the neuronal compartments and structures where energy metabolism is carried out *in vivo* (Villa et al., 2013b; Ferrari et al., 2015a,b), by this way assessing the effects of ADs on brain metabolism. In fact, the enzyme activities are: (i) indicative of the plastic properties of brain tissue to respond efficiently to drug treatments and pathological *noxae*; (ii) candidates as functional molecular targets for drug actions (Moretti et al., 2003, 2011, 2015); (iii) validated biochemical parameters to assess neuronal energy metabolism and transduction in Pathology and Pharmacology (Villa et al., 1992, 2013a, 2013b).

EXPERIMENTAL PROCEDURES

The experiments were performed on male Sprague–Dawley rats (Charles-River, Calco, Italy). The rats were housed under standard cycling and housing conditions, fed with a standard diet in pellets (VRF1, Charles River) specifically assembled for rats with water *ad libitum*.

Animal selection for pharmacological treatment was made by Fisher and Yates permutation tables, and the rats were divided in three experimental lots of 6–8 animals each for a total of 18–24 animals: (a) control animals treated with saline physiological solution; (b) animals treated with desipramine (desmethylimipramine) at the dose of 15 mg/kg b.w. per day, by intraperitoneal injection (i.p.); (c) animals treated with fluoxetine at 10 mg/kg b.w. per day, by i.p. The pharmacological treatment was started from the 7th week of age and continued for 21 days, taking into consideration the known time-lag between the pharmacological and therapeutical effect of these drugs.

At the end of treatments (10th week of age), the animals were sacrificed by anesthesia by ether at 09:00 a.m., after 24 h from the last drug administration, and the brain was isolated at the University of Modena and Reggio Emilia.

From the isolated brains, the frontal area of cerebral cortex was dissected according to Glowinsky and Iversen (1966) and frozen in liquid nitrogen (< 20 s) to subsequently perform the subfractionation techniques and the enzymatic analyses at University of Pavia. This study was approved by ad hoc authorities, following the guidelines of the Italian Ministry of Health.

At the time of experiment, the frontal cerebral cortex was thawed and immersed in the isolation medium according to Lai et al. (1977); subcellular fractionation was made as described by Villa et al. (1989, 2012a,b, 2013a).

On the different cerebral mitochondria, V_{max} of the following enzyme activities was evaluated: citrate synthase (CS) (EC 4.1.3.7) (Sugden and Newsholme, 1975), succinate dehydrogenase (SDH) (EC 1.3.99.1) (Ackrell et al., 1978), that is also part of the Electron Transport Chain (ETC) as Complex II, malate dehydrogenase (MDH) (EC 1.1.1.37) (Ochoa, 1955) for Krebs’ cycle; NADH-cytochrome *c* reductase (CCRT; CCRS) (EC 1.6.99.3) (Nason and Vasington, 1963); cytochrome oxidase (EC 1.9.3.1) (Smith, 1955; Wharton and Tzagoloff, 1967; Smith et al., 1974) for ETC; glutamate dehydrogenase (GLDH) (EC 1.4.1.3) (Sugden and Newsholme, 1975); glutamate-oxaloacetate transaminase (GOT) (EC 2.6.1.1) (Lai et al., 1977); glutamate-pyruvate transaminase (GPT) (EC 2.6.1.2) (Bergmeyer and Bernt, 1974) for glutamate and related amino acids metabolism, and the motility of amino groups.

The protein concentration of the tested subfractions was determined using crystalline bovine serum albumin as standard, according to Lowry et al. (1951).

Enzyme activities V_{max} were measured using a Perkin-Elmer 554 Spectrophotometer and were expressed as specific activities [S.A.: μmoles of substrate transformed) $\times$ min^{-1} $\times$ (mg of protein)$^{-1}$].

Because all the obtained data are homoscedastic and fit in the Gauss’ normal distribution, according to this experimental design, at first the homogeneity of variance was checked by Bartlett’s test, and afterward