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Doctoral Thesis Summary
(Autoreferát disertační práce)

Effects of antidepressants and depressive disorders on mitochondrial functions

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Content

Abbreviations

ETC - electron transport chain
MAO - monoamine oxidase
OXPHOS - oxidative phosphorylation
TCA - tricarboxylic acid cycle

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Abstrakt

Poruchy nálady jsou závažná onemocnění, přesto jejich patofyziologie není dostatečně objasněna a biologické markery jsou stále hledány. Cílem práce bylo zjistit, zda jsou vybrané mitochondriální funkce ovlivněny antidepresivy, stabilizátory nálady a depresivní poruchou. Předpokládáme, že narušená funkce mitochondrií vede k poškození neuronů, které může souviset se vznikem poruch nálady, a účinky antidepresiv a stabilizátorů nálady na mitochondriální funkce mohou být vztaženy k jejich výsledným terapeutickým účinkům.

Byly měřeny účinky farmakologicky odlišných antidepresiv a stabilizátorů nálady na mitochondrie izolované ze zvířecích mozků (*in vitro* model). Aktivita monoaminooxidasy (MAO) byla měřena radiochemicky, aktivita enzymů citrátového cyklu a respiračního řetězce byla stanovena spektrofotometricky. Celková aktivita systému oxidační fosforylace byla určena respirometrií s vysokým rozlišením.

Všechna testovaná antidepresiva inhibovala aktivitu MAO, přičemž se lišila inhibiční účinností, typem inhibice a specificitou pro izoformy MAO. Stabilizátory nálady aktivitu MAO neovlivňovaly. Všechna testovaná psychofarmaka zvyšovala nebo neměnila aktivitu citrátsyntasy a snižovala aktivitu komplexů elektronového transportního řetězce. Respirační rychlost byla antidepresivy inhibována, stabilizátory nálady téměř neovlivněna. Respirometrická technika byla modifikována pro měření v krevních destičkách u pacientů s depresivní epizodou a zdravých kontrol. Měření ukázala, že fyziologická respirace v destičkách depresivních pacientů není významně odlišná od kontrol; ke změnám ale dochází vlivem léčby.

Účinky antidepresiv a stabilizátorů nálady zahrnují výrazné změny aktivity mitochondriálních enzymů. Nejvíce byly ovlivněny MAO, citrátsyntasa, komplexy I a IV a respirační rychlost mitochondrií. Tyto mitochondriální parametry lze proto dále testovat jako potenciální biologické markery poruch nálady, cíle nově vyvíjených antidepresiv nebo prediktory účinnosti farmakologické léčby.

Abstract

Mood disorders are serious diseases, nevertheless, their pathophysiology is not sufficiently clarified and biological markers are still being sought. The aim of the study was to find out whether mitochondrial functions are affected by depression, antidepressants and mood stabilizers. It is suggested that impaired function of mitochondria leads to neuronal damage and can be related to the origin of mood disorders; effects of antidepressants and mood stabilizers can be associated with their mitochondrial effects.

Effects of pharmacologically different antidepressants and mood stabilizers on mitochondria isolated from pig brains (*in vitro* model) were studied. Monoamine oxidase (MAO) activity was measured radiochemically, activities of other mitochondrial enzymes and complexes of the electron transport chain (ETC) were assessed spectrophotometrically. Total activity of oxidative phosphorylation was measured electrochemically using high-resolution respirometry.

All antidepressants tested inhibited MAO activity; they differed in inhibitory potency, type of inhibition, and specificity for MAO isoforms. Mood stabilizers did not significantly affect MAO. Citrate synthase activity was increased or unaffected by all tested drugs; activities of ETC complexes were decreased. The respiratory rate was inhibited by antidepressants, and unaffected by mood stabilizers. Respirometry technique was modified to enable measurement in blood platelets of patients with depressive episodes and healthy controls. We found that physiological respiration in blood platelets of depressive patients did not differ from controls. Changes were induced by treatment with antidepressants.

Effects of antidepressants and mood stabilizers are comprised of marked changes in mitochondrial functions. MAO, citrate synthase, complexes I and IV, and respiratory rate of mitochondria were the most affected and are suggested as candidates in searching of new biological markers of mood disorders, targets of new antidepressants or predictors of response to pharmacotherapy.

1. Introduction

Mood disorders are serious diseases; nevertheless, their pathophysiology has not yet been sufficiently clarified. Biological markers that would facilitate the successful diagnosis and prediction of pharmacotherapy are still being sought.

Recently, attention in the research of a biological basis of mood disorders has been devoted to an overlapping set of molecular and cellular mechanisms of mood disorders, antidepressant response, neuroplasticity, and chronic stress (Pittenger and Duman, 2008), e. g. to changes in neuroprogression, inflammatory and cell-mediated immune response, antioxidant capacity, oxidative and nitrosative stress, and mitochondrial functions (Maes et al., 2012). Therefore, changes in the activities of the compounds of these intracellular signaling pathways are studied with the aim of discovering new biological markers of mood disorders or predictors of response to antidepressant treatment (Fišar and Raboch, 2008; Fišar and Hroudová, 2010). Mitochondrial dysfunctions are assuming an increasingly important role in hypotheses about mood disorders, mainly bipolar disorder. Recently discussed biological hypotheses of mood disorders include the neurotrophic and neuroplasticity hypothesis of depression (Duman et al., 1997; Duman, 2002; Zarate et al., 2006; Einat and Manji, 2006; Pittenger and Duman, 2008) and the mitochondrial hypothesis (Stork and Renshaw, 2005; Kato, 2008; Quiroz et al., 2008).

The majority of energy sources, necessary for cellular functions, originate from oxidative phosphorylation (OXPHOS) located in the inner mitochondrial membrane. Thus, the highest number of mitochondria is present in organs demanding the most of energy - brain, liver and muscles. According to chemiosmotic theory (Mitchell, 1961), most of ATP synthesis comes from the electrochemical gradient across the inner membranes of mitochondria by ATP synthase. The ATP production is regulated by many control mechanisms - firstly by mitochondrial membrane potential $\Delta\psi_m$, rate of coupling and proton leak. Recently, these mechanisms were

implemented by “second control mechanisms:” reversible phosphorylation of tricarboxylic acid cycle (TCA) enzymes and ETC complexes, allosteric inhibition of cytochrome *c* oxidase (COX), thyroid hormones, effects of fatty acids and uncoupling proteins.

In addition to their crucial role in generation of ATP, mitochondria are involved in other important processes, such as regulation of the free radicals, neurotransmitters, calcium, and apoptosis. Mitochondria strongly affect many intracellular processes coupled to signal transduction, neuron survival and plasticity. Impaired mitochondrial functions may be related to many psychiatric and neurodegenerative diseases, including bipolar disorder, major depressive disorder, schizophrenia, psychosis and anxiety (Shao et al., 2008; Rezin et al., 2009; Jou et al., 2009). There is increasing evidence that mitochondrial dysfunctions are associated with mood disorders (Hroudová and Fišar, 2011).

It is suggested that disturbed energetic metabolism and/or reactive oxygen species production take part in the pathophysiology of mood disorders and could participate in therapeutic or side effects of antidepressants and mood stabilizers. Variations in mitochondrial genome as well as defects of electron transport chain (ETC) complexes have been implicated in the pathogenesis of psychiatric diseases (Rollins et al., 2009; Zhang et al., 2008).

It is suggested that antidepressants and mood stabilizers cause changes in intracellular pathways and improve energy metabolism. Variability in intracellular processes probably participates in interindividual differences of the response to treatment with antidepressant or in drug resistance. Further studies of effects of mood disorders, antidepressants and mood stabilizers on the molecular level are necessary to understand their roles in signalling pathways and influences on energy metabolism of neurons. They are expected to be helpful both in the search of biological markers of mood disorders or predictors of efficiency of the treatment with antidepressants and in the search of new psychotropic drugs.

Impaired functions of mitochondria can be assessed both in isolated mitochondria and in intact or permeabilized cells. Better insight into molecular mechanisms of cellular respiration, control of oxidative phosphorylation (OXPHOS) and effects of antidepressants and mood stabilizers on these processes is likely to lead to a better understanding of the pathophysiology of neuropsychiatric disorders or interindividual variations in response to pharmacotherapy. Study of interactions between mitochondrial function, energy metabolism and neuronal activity are necessary for understanding physiological processes as well as pathophysiology of neuropsychiatric diseases (Kann and Kovács, 2007). Processes of OXPHOS are accompanied by increased production of reactive oxygen species, apoptosis, and participate in various diseases.

2. Hypothesis and aims of the study

The hypothesis was formulated that impaired energy metabolism of brain cells is concerned in the pathophysiology of mood disorders and in therapeutic and/or adverse effects of antidepressants. We presume that therapeutic effects of psychopharmaca, presently administered or newly developed for the treatment of depression, can be found in targeted regulation of mitochondrial functions and subsequent affection of neuroplasticity, inflammatory responses related to the disease, calcium homeostasis, production of reactive oxygen and nitrogen species and other processes related to the complex response to stress, neurotoxicity or impaired neurotransmission.

On the basis of this hypothesis, we have studied effects of depressive disorder, antidepressants and other psychoactive drugs with pharmacologically different mechanisms of action on the activities of mitochondrial enzymes.

The aim of our study has been specification of some intracellular biochemical parameters, which are affected by antidepressants and mood stabilizers and therefore can be related to the pathophysiology of mood

disorders. Based on results obtained from *in vitro* measurements and, consequently, from blood samples, we attempt to find biological markers of mood disorders that could help in the diagnosis and treatment as well as in the development of new drugs with specific effects on these newly discovered intracellular targets.

Thus, the primary aim of this study was discovery of the drug-induced changes in selected mitochondrial functions using antidepressants, mood stabilizers and animal brain mitochondria as an *in vitro* model. The subsequent aim was application of these findings to the preclinical research of mitochondrial dysfunctions in blood platelets of patients suffering from depression.

3. Materials and methods

We applied radiochemical, spectrophotometrical, fluorescence methods and high-resolution respirometry. Effects of pharmacologically different antidepressants and mood stabilizers on mitochondrial functions were initially measured as drug-induced changes in activities of mitochondrial enzymes, especially MAOs and enzymes of citric acid cycle and OXPHOS system.

Next, experiments were conducted on the facts that the bioenergetics function of mitochondria can be investigated by measurement of rate of ATP formation and efficiency of the process (Merlo-Pich et al., 2004) and that measurement of oxygen consumption and its sensitivity to substrates, uncouplers and inhibitors can be a good indication of mitochondrial phosphorylation capacity.

Effects of depressive disorder on the activity of mitochondrial enzymes and on mitochondrial respiration were measured using human platelets.

3.1 Animal brain mitochondria

Isolated mitochondria from animal brains served as proper *in vitro* models for study of mitochondrial functions. They were prepared by a standard differential centrifugation method (Whittaker, 1969). The final

pellet containing mitochondria was resuspended to a protein concentration of 20-40 mg/ml, and either immediately used for measurement of respiratory rate, or stored at -70°C until the enzyme assayed. Protein concentration was determined by the method of Lowry et al. (1951), with bovine serum albumin as the standard.

3.2 Drug effects on mitochondrial enzymes

Crude mitochondrial fraction was resuspended with a hypotonic buffer. The suspension was frozen and thawed two times to achieve maximum enzyme activities (Kirby et al., 2007). Samples were incubated with selected psychopharmaca for 30 minutes at 30 ºC. Effects on the activities of enzymes in TCA (citrate synthase, succinate dehydrogenase) and enzymes in ETC (complexes I, II, IV) were determined spectrophotometrically (Srere, 1969; Rustin et al., 1994; Trounce et al., 1996; Folbergrová et al., 2007; Hroudova and Fisar, 2010).

MAO activity was determined radiochemically (Ekstedt, 1976; Egashira et al., 1978; Ozaita et al., 1997; Egashira et al., 1999; Fišar et al., 2010) with either radiolabelled serotonin (for MAO-A) or phenylethylamine (for MAO-B) as substrates. Antidepressants and mood stabilizers were compared with effects of well-known MAO inhibitors such as moclobemide, iproniazid, pargyline, and clorgyline. Steady-state kinetic constants (K_m, Michaelis constant and V_{max}, maximum rate) were determined from studies of the effects of substrate concentration on the initial reaction rate of MAO-A or MAO-B in the absence of drugs and in the presence of different concentrations of drugs.

3.3 Drug effects on mitochondrial respiratory rate

The respiratory rate of mitochondria, i.e. the total activity of the OXPHOS system, was measured in mitochondria as kinetic study of oxygen consumption using respirometer (Oxygraph-2k, Oroboros Instruments) with Clark-type electrodes. We measured effects on the respiratory state controlled by malate and pyruvate (complex I substrates), and the respiratory state controlled by succinate (complex II substrate). Achieved

results were implemented to suggest an experimental method that enabled the search of mitochondrial respiratory rate in blood platelets obtained from patients who suffered from depression.

3.4 Respiratory rate in platelets of depressive persons

Patients with major depressive disorder were submitted for the study at the beginning of treatment of a recurrent depressive episode and after several weeks of treatment with antidepressants. Severity of depression was assessed by the Hamilton Rating Scale for Depression (HRSD-21), the Clinical Global Impression - Severity scale (CGI-S) and the Clinical Global Impression - Improvement scale (CGI-I). The control group consisted of 16 healthy volunteers.

Activities of the respiratory system were analyzed in Oxygraph-2k (Oroboros Instruments, Innsbruck, Austria) at 37°C. The method of measurement in blood platelets was adapted (Sjövall et al., 2010), i.e. multiple substrate-uncoupler-inhibitor titration protocols for high-resolution respirometry were developed for accurate measurement with both intact and permeabilized platelets. Data were collected and analyzed using the software displaying the oxygen flux and real-time oxygen consumption (DatLab software 4.3b, Oroboros Instruments, Austria). O_2 flow per cell and flux control ratios were evaluated.

3.5 Data analysis

All data presented are expressed as the mean ± standard deviation. Results were analyzed by STATISTICA (StatSoft, Inc.). The Wilcoxon matched pairs test was applied in the *in vitro* model. This nonparametric test for dependent samples was used to calculate statistics in order to compare the enzyme activities in samples with and without the drug. Mann-Whitney U test was used to calculate test statistics in order to compare values obtained from patients with depressive episodes to values obtained from patients after long-term treatment and controls.

4. Results

4.1 Drug effects on mitochondrial enzymes' activities

Most of the antidepressants and mood stabilizers inhibited the activities of respiratory ETC complexes, but complexes I and IV were the most affected. A statistically significant decrease of the complex I activity was caused by desipramine, amitriptyline, imipramine, citalopram, mirtazapine, valproate and olanzapine. Complex II was significantly inhibited only by amitriptyline, imipramine, citalopram and venlafaxine. Complex IV was significantly inhibited by all tested drugs except for citalopram and moclobemide. Unchanged or slightly increased citrate synthase activity was observed; significant activation of the enzyme was observed after citalopram, tianeptine and olanzapine. Succinate dehydrogenase was significantly decreased only by amitriptyline.

Drug effects on both isoforms of MAO were found inhibitory. Various antidepressants and mood stabilizers differed both in inhibitory potency and in the type of inhibition for two isoforms. The half maximal inhibitory concentration, parameters of enzyme kinetic, and the mechanism of inhibition were determined. MAO-A was inhibited by the following drugs: pargyline > clorgyline > iproniazid > fluoxetine > desipramine > amitriptyline > imipramine > citalopram > venlafaxine > olanzapine > reboxetine > mirtazapine > tianeptine > moclobemide >> lithium, valproate. MAO-B was inhibited by the following drugs: pargyline > clorgyline > iproniazid > fluoxetine > venlafaxine > amitriptyline > olanzapine > citalopram > desipramine > reboxetine > imipramine > tianeptine > mirtazapine >> moclobemide, lithium, valproate. The mechanism of inhibition of MAOs by several antidepressants was found to be varied (competitive, noncompetitive, uncompetitive, mixed).

4.2 Drug effects on the OXPHOS system

Antidepressants, but not mood stabilizers, were found to inhibit the mitochondrial respiratory rate. The inhibitory potency of drugs was determined for electron supply through complex I: fluoxetine = tianeptine >

chlorpromazine > amitriptyline > ketamine; and for electron supply through complex II: tianeptine > fluoxetine = chlorpromazine > amitriptyline > olanzapine. The effective dose of antidepressants reaching half the maximal respiratory rate was in the rage of 0.07 to 0.46 mmol/l. Partial inhibition was found for all inhibitors.

4.3 Respiratory rate in blood platelets

The measurement techniques were consequently modified to measure the same mitochondrial parameters (respiratory rate and mitochondrial enzyme activities) in blood platelets of depressive patients before and after treatment and in controls.

In intact platelets from depressive subjects, physiological respiration and maximal ETC capacity were unchanged before the treatment, but were decreased after the treatment compared to controls. The ratio of physiological respiration to the maximal capacity of respiration, which characterizes the efficiency of the respiratory system, was significantly increased both before treatment and after treatment compared to healthy controls. Similar effects were observed in permeabilized platelets.

5. Discussion

Biological markers of depression and predictors of the response upon drug administration are being searched for on the basis of recently developed hypotheses of affective disorders, including the mitochondrial hypothesis. The leading role of mitochondrial dysfunctions in the pathophysiology of mood disorders and the effects of antidepressants was supported by our results.

The effects of selected antidepressants and mood stabilizers on mitochondrial functions were examined in pig brain mitochondria. The pig is a relatively unusual species for most pharmacological studies; however, pig mitochondria are relatively often used in studies of mitochondrial functions.

Citrate synthase, a rate-limiting enzyme of the TCA cycle, plays a decisive role in regulating energy generation of mitochondrial respiration. Most of the tested drugs increased citrate synthase activity, although the effect was not significant in all cases. The drug-induced decrease of enzyme activity was found to be statistically significant for complexes I and IV. Complex I is a rate limiting enzyme for oxygen consumption in synapses and plays a major role in controlling OXPHOS (Telford et al., 2009). Our data are consistent with previous data about the role of complex I in mental disorders and in mechanisms of action of psychotropic drugs (Wang, 2007). Complex I plays a major role in controlling OXPHOS and its abnormal activity can lead to defects in energy metabolism and thereby to changes in neuronal activity (Pathak and Davey, 2008). The neuroanatomical pattern of complex I pathology parallels the diversity and similarities in clinical symptoms of schizophrenia, major depressive disorder and bipolar disorder (Ben-Shachar and Karry, 2008).

Complex II is directly involved in the TCA cycle (Tomitsuka et al., 2009) and encoded only by nuclear DNA. Complex IV was suggested as an endogenous metabolic marker for neuronal activity (Wong-Riley, 1989). However, major role is given to complex I in controlling mitochondrial OXPHOS as a crucial point of respiration; its abnormality can result in mitochondrial dysfunction (Davey et al., 1998). We found drug-induced statistically significant decrease of complex I and IV activity for all tested antidepressants and mood stabilizers. Complex II activity was only slightly affected by drugs tested. Our data are consistent with previous data about the role of complex I in mental disorders and in mechanisms of action of psychotropic drugs (Wang, 2007).

The experimental conditions of the present study and the use of the selective substrates (radioactively labeled serotonin for MAO-A and phenylethylamine for MAO-B) allowed the inhibitory effects and mechanism of the interactions of antidepressants on the two MAO isoforms to be evaluated separately. Enzyme inhibition represents one of the most

common strategies in the development of therapeutic drug candidates. Estimation of an enzyme inhibitor binding affinity is an important step in predicting *in vivo* potency, selectivity, and potential for metabolic interactions. MAO inhibition is not the primary biochemical effect related to therapeutic action of the tested drugs; it can be supposed that a decrease of MAO activity may be concerned in some effects of these drugs on serotonergic, noradrenergic, and dopaminergic neurotransmission.

Antidepressant-induced decreases of the respiratory rate can be associated with adverse effects of pharmacotherapy with antidepressants. However, the possibility is not excluded that an antidepressant-induced decrease of the respiratory rate is the initial event in a complex cellular response to antidepressants in the intracellular milieu, leading to adaptive changes and finally to support of neuroplasticity. Thus, the hypothesis should be tested that weak antimitochondrial actions of antidepressants could provide a potentially protective pre-conditioning effect (Calabrese et al., 2010; Abdel-Razaq et al., 2011), in which antidepressant-induced mitochondrial dysfunction below the threshold of injury results in subsequent protection.

Physiological respiration and maximal capacity of ETS in intact platelets were significantly decreased in depressive patients after the treatment but not before treatment compared to controls. Net physiological respiration/ETS capacity ratio was significantly increased in depressive patients both before treatment and after the treatment compared to healthy controls. Higher flux control ratios indicate higher percent of utilization of respiratory system in depression; we hypothesize that it reflects adaptive response to increased cellular stress during depression. We suppose that decrease of respiratory rate in depressed subjects after treatment reflects inhibitory effect of antidepressants, which was observed in our *in vitro* experiments. However, a systematic *in vivo* investigation of the

antidepressants' or mood stabilizers' effects on the respiratory rate is necessary to confirm this clinically important conclusion.

6. Conclusions

Our findings of mitochondrial changes induced by antidepressants and mood-stabilizing drugs support the suggestion that mitochondrial dysfunction could be a primary event in mood disorders. However, it remains to be determined if mitochondrial dysfunction is rather a causal or a consequential event of abnormal signalling, and if effects of antidepressants and mood stabilizers on mitochondrial functions are related rather to therapeutic or to adverse effects of pharmacotherapy.

In vitro results comprised marked drug-induced changes of mitochondrial enzymes activities. Differences in inhibitory potency and in mechanism of inhibition were found between several drugs. Antidepressants, but not mood stabilizers, seem to be potent partial inhibitors of mitochondrial respiration supported both through complex I and complex II of ETC. MAOs, citrate synthase and complexes I and IV were the most affected mitochondrial enzymes and they can be suggested as proper candidates in search of new biological markers of mood disorders, targets of new pharmaca or predictors of response to pharmacotherapy. The effect of newly synthetized psychotropic drugs on mitochondrial respiration should be included in their testing to discover their mitochondrial toxicity or potential neurotrophic effects; high-resolution respirometry is a suitably sensitive technique for these measurements.

Multiple substrate-uncoupler-inhibitor titration protocols for high-resolution respirometry are useful for the sensitive and accurate measurement of the respiratory rate in both intact and permeabilized human platelets. Mitochondrial respiration is affected by antidepressants significantly more than by depressive disorder in itself. The relationship of antidepressant-induced changes in cellular respiration to the adverse or therapeutic effect of pharmacotherapy needs to be explored.

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List of publications

1. Publications *in extenso* related to this thesis

A) with IF

- **Hroudova J.**, Fisar Z.: Activities of respiratory chain complexes and citrate synthase influenced by pharmacologically different antidepressants and mood stabilizers. Neuroendocrinology Letters 2010; 31(3): 336-342 (IF 2010 = 1.621)
- Fišar Z., **Hroudová J.**: Intracellular signalling pathways and mood disorders. Folia Biol. 2010; 56(4): 135-148 (IF 2010 = 0.729)
- Fišar Z., **Hroudová J.**, Raboch J.: Inhibition of monoamine oxidase activity by antidepressants and mood stabilizers. Neuroendocrinology Letters 2010; 31(5): 645-656 (IF 2010 = 1.621)
- **Hroudová J.**, Fišar Z.: Connectivity between mitochondrial functions and psychiatric disorders. Psychiatry and Clinical Neurosciences 2011; 65(2): 130-141 (IF 2010 = 1.559**)**
- **Hroudová J.**, Fišar Z.: *In vitro* inhibition of mitochondrial respiratory rate by antidepressants. 2012; in press

B) without IF

- Fišar Z., **Hroudová J.**: Common aspects of neuroplasticity, stress, mood disorders and mitochondrial functions. Act. Nerv. Super. Rediviva. 2010; 52(1): 3-20.
- Fišar Z., **Hroudová J.**, Raboch J.: Neurotransmission in Mood Disorders. In: Clinical, Research and Treatment Approaches to Affective Disorders, Mario Francisco Juruena (Ed.), InTech, Rijeka, Croatia, 2012, pp. 191-234. Available from: http://www.intechopen.com/articles/show/title/neurotransmission-in-mood-disorders.
- Fišar Z., **Hroudová J.**., Raboch J.: Úloha mitochondrií v mechanismech synaptické plasticity, buněčného poškození a poruch nálady. Česká a slovenská psychiatrie. 2011; 107(1): 14-27.
- Fišar Z., **Hroudová J.**, Raboch J.: Biochemické mechanismy účinků antidepresiv. Časopis lékařů českých. 2011; 150(10): 531-540.

2. Publications *in extenso* unrelated to this thesis

A) with IF

- Korábecny J., Musilek K., Holas O., Zemek F., Opletalova V., Dohnal V., Nachon F., **Hroudova J.**, Fisar Z., Kuca K.: Synthesis and *In Vitro* Evaluation of *N*-(bromobut-3-en-2-yl)-7-methoxy-1,2,3,4-tetrahydroacridin-9-amine as Cholinesterase Inhibitor with regard to Alzheimer's disease treatment. Molecules 2010; 15(12): 8804-8812 (IF 2010 = 1.988)
- **Hroudová J.**, Fišar Z., Korábečný J., Kuča K.: *In vitro* effects of acetylcholinesterase inhibitors and reactivators on Complex I of electron transport chain. Neuroendocrinology Letters 2011; 32(3): 259-263 (IF 2010 = 1.621)
- Fišar Z., **Hroudová J.**, Korábečný J., Musílek K., Kuča K. *In vitro* effects of acetylcholinesterase reactivators on monoamine oxidase activity. Toxicology Letters 2011; 201(2): 176-180 (IF 2010 = 3.581)
- Lang M.F., Salinin S., Ridder D.A., Kleesiek J., **Hroudova J.**, Berger S., Schűtz G., Schwaninger M. A transgenic approach to identify thyroxine transporter-expressing in brain development. Journal of Neuroendocrinology 2011; 23(12): 1194-1203 (IF 2010 = 4.650)
- Korabecny J., Musilek K., Zemek F., Horova A., Holas O., Nepovimova E., Opletalova V., **Hroudova J.**, Fisar Z., Jung Y.S., Kuca K.: Synthesis and in vitro evaluation of 7-methoxy-*N*-(pent-4-enyl)-1,2,3,4-tetrahydroacridin-9-amine–new tacrine derivate with cholinergic properties. Bioorg Med Chem Lett. 2011; 21(21): 6563-6566 (IF 2010 = 2.661)
- Kitzlerová E., Fišar Z., Roman J., Zvěřová M., **Hroudová J.**, Benáková H., Raboch J.: Relationship of plasma homocysteine with depression and progression of Alzheimer's disease. 2012; in press