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Effects of antidepressants and depressive disorders on mitochondrial functions

Vliv antidepresiv a depresivní poruchy na mitochondriální funkce

Dissertation Thesis

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Abbreviations

ADP - adenosine 5´-diphosphate

AIF - apoptosis inducing factor

ANT - adenine nucleotide translocator

Apaf-1 - apoptotic protease-activating factor-1

ATP - adenosine 5´-triphosphate

α-KGDH - α-ketoglutarate dehydrogenase

Bcl-2 - B-cell CLL/lymphoma 2

BDNF - brain-derived neurotrophic factor

BSA - bovine serum albumin

CNS - central nervous system

COX - cytochrome *c* oxidase

CoQ - coenzyme Q_{10}, ubiquinone

CREB - cAMP response element binding protein

CS - citrate synthase

cyt *c* - cytochrome *c*

DALY - Disability Adjusted Life Year

DCPIP - 2,6-dichlorophenolindophenol

DNTB - 5,5´-dithiobis-(2-nitrobenzoic) acid

ETC - electron transport chain

ETS - electron transfer system

FA - fatty acid

FAD - flavin adenine dinucleotide

FCCP - *p*-(trifluoromethoxy)phenylhydrazone

FCR - flux control ratio

5-HT - 5-hydroxytryptamine, serotonin

K_i - dissociation constant of an enzyme-inhibitor complex

K_m - Michaelis constant

LEAK - state 4, non-phosphorylating resting state, limited by low ADP

IC_{50} - half maximal inhibitory concentration

MAO - monoamine oxidase

MAOI - monoamine oxidase inhibitor

MAPK - mitogen-activated protein kinase

MiR05 - mitochondrial respiration medium

MRS - magnetic resonance spectroscopy

MPP^+ - 1-methyl-4-phenylpyridinium

MPT - mitochondrial permeability transition

MPTP - mitochondrial permeability transition pores

mtDNA - mitochondrial DNA

NADH - reduced nicotinamide adenine dinucleotide

NaSSA - noradrenergic and specific serotonergic antidepressant

NDRI - norepinephrine-dopamine reuptake inhibitor

NGF - nerve growth factor

NRI - norepinephrine reuptake inhibitor

nDNA - nuclear DNA

NE - norepinephrine

NMDA - *N*-methyl-D-aspartic acid

OXPHOS - oxidative phosphorylation

PEA - 2-phenylethylamine hydrochloride

PET - positron emission tomography

PKC - protein kinase C

ROS - reactive oxygen species

SDH - succinate dehydrogenase

SH-SY5Y – human neuroblastoma cells

SNRI - serotonin-norepinephrine reuptake inhibitor

SSRE - selective serotonin reuptake enhancer

SPECT - single-photon emission computed tomography

SSRI - selective serotonin reuptake inhibitor

TCA - tricarboxylic acid cycle

TTFA - thenoyltrifluoroacetone

V_{max} - maximum rate of enzymatic reaction

VDAC - voltage-dependent anion channel

YLDs - Years Lived with Disability

$\Delta\psi_m$ - mitochondrial membrane potential

Δp - proton motive force

Content

Abstrakt

Poruchy nálady jsou závažná onemocnění, přesto jejich patofyziologie nebyla dostatečně objasněna. Biologické markery, které by zlepšily diagnostiku onemocnění nebo predikci úspěšné farmakoterapie, jsou hledány. Cílem práce bylo zjistit, zda jsou vybrané mitochondriální funkce ovlivněny antidepresivy, stabilizátory nálady a depresivní poruchou. Výzkum vychází z hypotéz poruch nálady a mechanismů účinků antidepresiv, ze zdokonalené monoaminové hypotézy, neurotrofní hypotézy a hypotézy o mitochondriální dysfunkci. Narušená funkce mitochondrií vede k poškození neuronů, které může souviset se vznikem poruch nálady. Účinky antidepresiv a stabilizátorů nálady na mitochondriální funkce mohou být vztaženy k jejich terapeutickým či vedlejším účinkům.

Byly měřeny účinky farmakologicky odlišných antidepresiv a stabilizátorů nálady na mitochondrie izolované ze zvířecích mozků (*in vitro* model). Aktivita izoforem monoaminooxidasy (MAO) byla stanovena radiochemicky, aktivity dalších mitochondriálních enzymů spektrofotometricky. Celková aktivita systému oxidační fosforylace byla určena respirometrií s vysokým rozlišením. Metody byly modifikovány pro měření stejných parametrů v krevních destičkách u pacientů s depresivní epizodou a u zdravých kontrol.

Všechna testovaná antidepresiva inhibovala aktivitu MAO, lišila se rozdílnou inhibiční účinností, typem inhibice a specificitou pro izoformy MAO. Stabilizátory nálady aktivitu MAO neovlivňovaly. Všechny testované látky zvýšily nebo neovlivnily aktivitu citrátsyntasy (CS). Aktivita komplexů elektronového transportního řetězce (ETC) byla snížena, nejvýrazněji u komplexů I a IV. Respirační rychlost byla testovanými antidepresivy při vyšších koncentracích inhibována, stabilizátory nálady, olanzapin a ketamin ji téměř neovlivňovaly.

Fyziologická respirace v destičkách depresivních pacientů se nelišila od kontrol; ke snížení došlo po léčbě antidepresivy. Maximální kapacita ETC byla snížena u depresivních pacientů před i po léčbě antidepresivy. Poměr fyziologické respirace k maximální kapacitě respirace byl významně zvýšen před léčbou.

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. Biological markers that would facilitate the diagnosis or successful prediction of pharmacotherapy are still being sought. The aim of the study was to find out whether mitochondrial functions are affected by antidepressants, mood stabilizers and depression. Our research is based on recent hypotheses of mood disorders, the advanced monoamine hypothesis, the neurotrophic hypothesis, and the mitochondrial dysfunction hypothesis. We assume 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 on mitochondrial functions can be related to their therapeutic or side effects.

In vitro effects of pharmacologically different antidepressants and mood stabilizers on the activities of mitochondrial enzymes were measured in mitochondria isolated from pig brains (*in vitro* model). Activity of monoamine oxidase (MAO) isoforms was determined radiochemically, activities of other mitochondrial enzymes were measured spectrophotometrically. Overall activity of the system of oxidative phosphorylation was measured electrochemically using high-resolution respirometry. Methods were modified to measure the same parameters in blood platelets of patients with depressive episode and healthy controls.

Though all antidepressants tested inhibited MAO activity, they differed in inhibitory potency, type of inhibition, and specificity for two isoforms. Mood stabilizers did not affect MAO. All drugs tested increased or left citrate synthase (CS) activity unchanged. Activity of electron transport chain (ETC) complexes was decreased. The most affected were complexes I and IV. While respiratory rate of mitochondria was inhibited at higher concentrations of antidepressants, it was not affected by mood stabilizers, olanzapine and ketamine.

Physiological respiration in the blood platelets of depressive patients did not differ from controls; decrease was observed after treatment with antidepressants. Maximal capacity of ETC was decreased both before and after treatment with antidepressants. Ratio of physiological respiration to maximal capacity was significantly increased before the treatment.

Effects of antidepressants and mood stabilizers are comprised of marked changes in mitochondrial functions. MAO, CS, 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

Depression is a serious mental disorder manifested by depressed mood, pessimistic thoughts, feelings of worthlessness, feelings of guilt, tearfulness, reduced or increased sleep, appetite loss or appetite disturbance, weight loss or weight gain, social restlessness, loss of interest, difficulty concentrating. Mania is characterized by abnormally elevated or irritable mood, arousal, and/or energy levels. Bipolar disorder features intermittent episodes of mania or hypomania and depressive episodes; rapid cycling; mixed states; and psychotic symptoms, occurring in some cases. Depression and mania are thought to be heterogeneous illnesses that can result from dysfunction of several neurotransmitters or metabolic systems.

The estimated lifetime prevalence of mood disorders varies from 3.3 to 21.4 % in several countries (Kessler et al., 2007). The World Health Organization currently ranks depression as the leading cause of disability as measured by YLDs (Years Lived with Disability) and the 4th leading contributor to the global burden of disease (Disability Adjusted Life Years, DALYs) in 2000. By the year 2020, depression is projected to reach the 2nd place of the ranking of DALYs (World Health Organization Web site: Depression. http://www.who.int/mental_health/ management/depression/definition/en/). The predisposition to the disease is determined by genetic, psychosocial and biological factors; individual sensitivity to depressogenic effects during stressful life events is also a contributing factor. Pathophysiology of mood disorders is not sufficiently elucidated and about 1/3 of patients do not response to pharmacotherapy sufficiently. The exact molecular site and the primary cause of signal transduction disturbance associated with the symptoms of depression or mania are still unknown.

Recently, attention in the research of 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 compounds of these intracellular signalling 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, 2010b). Mitochondrial dysfunctions are assuming an increasingly important role in hypotheses of mood disorders,

bipolar disorder mainly. 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).

It is well-known that mitochondria strongly affect many intracellular processes coupled to signal transduction, neuron survival and plasticity. Impaired mitochondrial functions manifest themselves in various ways, they 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; Orth and Schapira, 2001; Schapira, 2012). 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 pathophysiology of neuropsychiatric disorders.

1.1 Mitochondria

Mitochondria are small cellular structures consisting of an outer and inner membrane, an intermembrane space and an intracellular matrix. The outer membrane covers the organelle, the inner membrane folds and forms cristae. This settlement extends the surface and enables plenty of chemical reactions. In the mitochondrial matrix, the enzymes of the tricarboxylic acid cycle (TCA, also called citric acid cycle or Krebs cycle) are localized. It is the central pathway of metabolism; its main function is oxidation of acetyl-CoA derived from carbohydrates, amino acids and fatty acids (FAs). The TCA is organized into a supramolecular complex that enables interaction with mitochondrial membranes and the electron transport chain (ETC) in OXPHOS (Vélot and Srere, 2000). Most of the TCA enzymes provide other additional “moonlighting” functions, e.g. they stabilize the mitochondrial DNA (mtDNA) or are associated with mitochondrial RNA (mtRNA) translation, oxidative stress, iron metabolism and tumour suppression (Sriram et al., 2005).

In addition to their crucial role in generation of adenosine-5’- triphosphate (ATP), mitochondria are involved in other important processes, such as regulation of free radicals,

neurotransmitters, calcium, and apoptosis. They are also involved in neuronal development - synaptogenesis, synaptic development and plasticity. Impaired function of mitochondria leads to impaired bioenergetics, decrease of ATP production, impaired calcium homeostasis, increased production of free radicals and oxidative stress (Fišar and Hroudová, 2010a; Hroudová and Fišar, 2011). Furthermore, monoamine oxidase (MAO), the enzyme responsible for the metabolism of monoamine neurotransmitters, is localized in the external mitochondrial membrane.

Mitochondrial proteins are encoded by both nuclear and mitochondrial DNA. All 13 polypeptides encoded by mtDNA form subunits of respiratory chain complexes I, III, IV and V (Scheffler, 1998; Müller et al., 2005). Furthermore, the mitochondrial genome encodes transfer RNA (tRNA) and ribosomal RNA (rRNA) used for RNA translation (Reinecke et al., 2009). Complex II is encoded only by nuclear DNA (nDNA). OXPHOS is under the control of the nuclear genome as well as the mitochondrial genome, which is only maternally inherited. Nevertheless, the dominant role in the regulation of mitochondrial activity has a nucleus; nuclear-encoded transcript factors control the activity of the mitochondrial genome and coordinate the expression of nuclear and mitochondrial genes to mitochondrial proteins (Cannino et al., 2007; Reinecke et al., 2009).

Genetic defects or stress can cause mitochondrial dysfunctions, which leads to increased oxidative stress and/or altered calcium homeostasis (Hovatta et al., 2010). An excess of glutamate in the synapse (Moghaddam, 2002) leads to an excess of cytosolic calcium, which produces overactivity of calcium-dependent enzymes and an overload of mitochondria by calcium; it leads to cytoskeletal degradation, protein malformation, decrease of ATP production and increase of oxygen radical generation. These processes can lead to atrophy or death of neurons (Atlante et al., 2001; Lipton, 1999). Different impulsions, such as hypoxia-ischemia, seizure and hypoglycemia, all activate this pathway. Thus, enhancing mitochondrial function may represent a critical component for the optimal treatment of stress-related diseases (Quiroz et al., 2008).

Eukaryotes synthetize ATP mainly by glycolysis in the cytosol and by OXPHOS in the mitochondria; i.e. the majority of cellular ATP is generated by glycolytic degradation of glucose to pyruvate in cytosol followed by aerobic cellular respiration. When pyruvate is converted to acetyl coenzyme A (acetyl-CoA), acetyl-CoA enters the TCA cycle and the result of this process is ATP production by OXPHOS in mitochondria (Ludwig et al., 2001). OXPHOS yields about 17

times more ATP than glycolysis. Therefore, it is considered as the main energy source and a key element of bioenergetics (Gnaiger, 2009; Kadenbach et al., 2010). Integration of main metabolic pathways coupled to OXPHOS is illustrated in Fig. 1.

The highest number of mitochondria is present in organs demanding the most energy - brain, liver and muscles. Neurons usually utilize glucose as a source of energy. Since the brain stores only a very small amount of glycogen, it needs a steady supply of glucose. Neurons are known to have a lower glycolytic rate than astrocytes and when stressed they are unable to upregulate glycolysis. Following inhibition of mitochondrial respiration, neurons die rapidly, whereas astrocytes utilize glycolytically generated ATP. Glucose metabolism in neurons is directed mainly to the pentose phosphate pathway, leading to regeneration of reduced glutathione, which probably supports antioxidant controlled neuron survival (Bolaños et al., 2010). The regulative processes of OXPHOS are tightly related to reactive oxygen species (ROS) production, integrity of mitochondrial membranes, apoptosis, and intramitochondrial Ca^{2+} levels. Although this is known, the control mechanisms have not yet been sufficiently investigated.

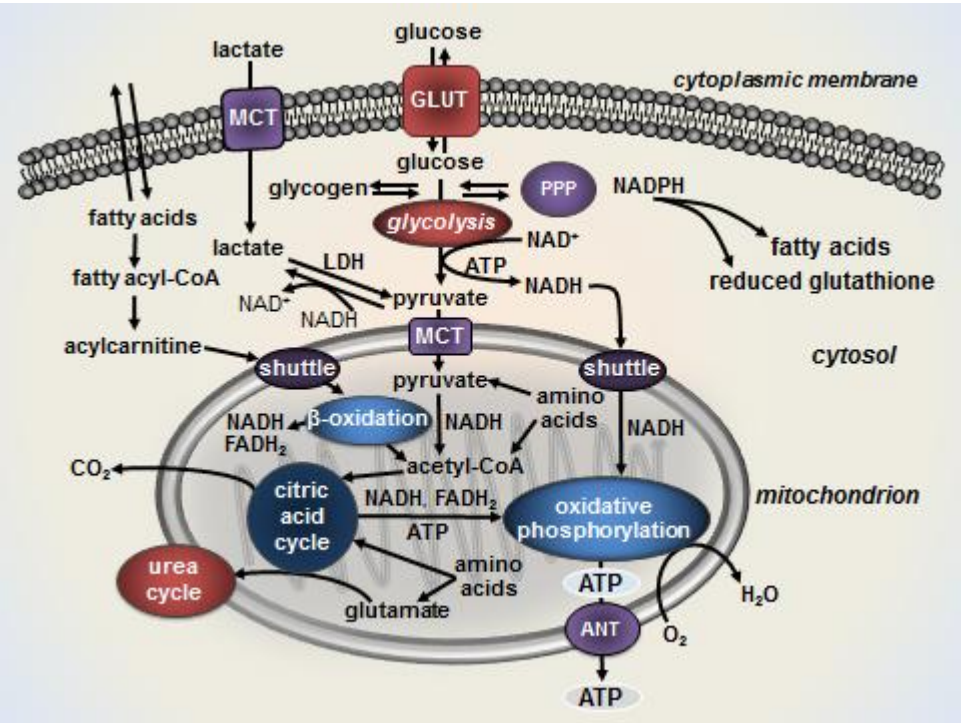


Fig. 1 Integration of metabolic pathways. Glucose is transported over a plasma membrane by a glucose transporter (GLUT) and is metabolized to pyruvate by glycolysis. Pyruvate is converted to acetyl-coenzyme A (acetyl-CoA) in the mitochondria, where it is oxidized to CO_2 through the citric acid cycle; redox energy is conserved as reduced nicotinamide adenine dinucleotide (NADH). The Mitochondrial respiratory chain couples

NADH oxidation to the formation of the electrochemical proton gradient across the inner mitochondrial membrane, which is used to form ATP. ATP produced from OXPHOS is transported from the mitochondrial matrix to the cytoplasm by the adenine nucleotide translocator (ANT). Glucose may be stored as glycogen. Fatty acids (FAs) and amino acids can also be bioenergetics precursors; however, glucose is considered to be the only metabolic substrate in the brain. Glucose can also be metabolized via the pentose phosphate pathway (PPP), a process that generates pentoses and that is the most important cytosolic source of reduced nicotinamide adenine dinucleotide phosphate (NADPH), a cofactor for biosynthetic reactions and the oxidation-reduction involved in protecting against the oxidative stress, e.g. for FA biosynthesis or regeneration of reduced glutathione. During activation the brain may transiently turn to aerobic glycolysis occurring in astrocytes, followed by the oxidation of lactate by neurons (Magistretti and Pellerin, 1999; Bolaños et al., 2010). Monocarboxylate transporters (MCTs) carry lactate or pyruvate across biological membranes; lactate dehydrogenase (LDH) catalyzes the interconversion of pyruvate and lactate with concomitant interconversion of NADH and NAD^+.

1.1.1 Physiology of oxidative phosphorylation

The respiratory chain is localized in cristae, structures formed by the inner mitochondrial membrane and extending to the surface (Vonck and Schäfer, 2009). ETC consists of complexes with supramolecular organization, where mitochondrial proton pumps (complexes I, III and IV) transport protons and generate a proton gradient (Bunoust et al., 2005; Kadenbach et al., 2010). Continuously, electrons are transported to complex III and finally complex IV enables the conversion of O_2 to H_2O. Most of the ATP synthesis comes from the electrochemical gradient across the inner membranes of mitochondria by ATP synthase (complex V). The CoQ cofactor is responsible for transferring electrons from complexes I and II to complex III; the second important cofactor is cytochrome *c* (cyt *c*), which transfers electrons from complex III to complex IV (Solmaz and Hunte, 2008). Both cofactors modulate energy and free radical production (Rodríguez-Hernández et al., 2009; Mattson et al., 2008). Processes in the inner mitochondrial membrane are depicted in Fig. 2.

Energy saved in ATP is used in synaptic ion homeostasis and phosphorylation reactions. ATP is essential for the excitability and survival of neurons, OXPHOS is involved in synaptic signalling and is related to changes of neuronal structure and function. Therefore, mitochondria are included in neurotransmitter exocytosis, in recovery, and in ion homeostasis, and in presynaptic nerve terminals.

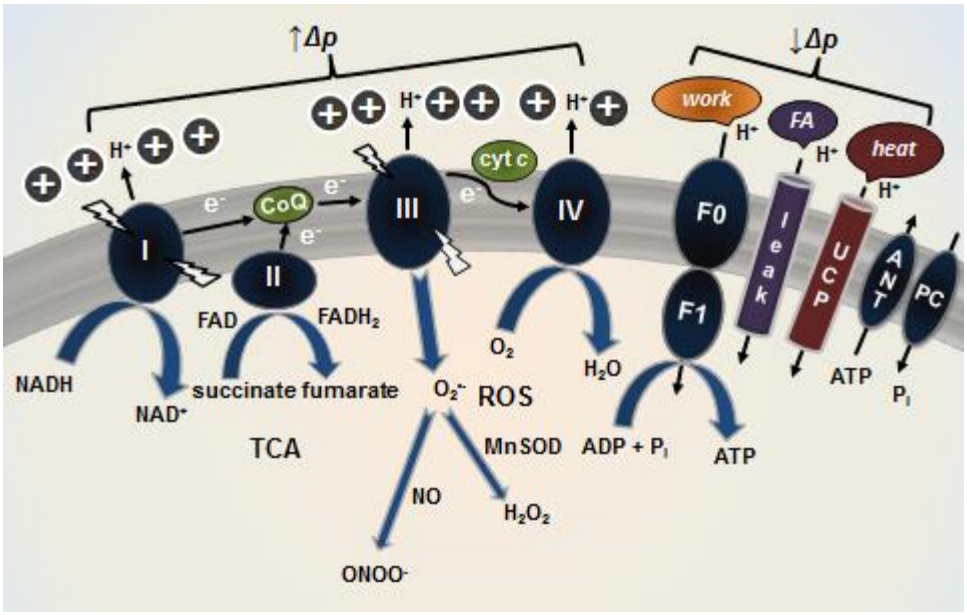


Fig. 2 Representation of processes in the inner mitochondrial membrane. ETC consists of I - IV complexes that transfer electrons, pump protons outwardly, and create proton motive force (*Δp*). Complex I catalyzes oxidation of nicotinamide adenine dinucleotide (NADH), complex II oxidizes succinate to fumarate. CoQ as a cofactor accepts electrons from complexes I and II, and carries them to complex III; the second mobile carrier cyt *c* move electrons from complex III to complex IV (COX), where O_2 is finally reduced to water. The proton gradient is primarily consumed by F_0F_1 ATP synthase for ATP synthesis from ADP and inorganic phosphate P_i. Secondary consumers causing decreased *Δp* are uncoupling proteins (UCPs), they response to heat production, proton leak is mediated e.g. by FAs. Transport of ADP and ATP across the membrane is enabled by adenine nucleotide translocator (ANT); mitochondrial phosphate carrier protein (PC) catalyzes movement of P_i into the mitochondrial matrix. Simultaneously, electron transport is accompanied by generation of reactive oxygen species (ROS), the highest amount of superoxide ($O_2^{\bullet-}$) is formed by complexes I and III. $O_2^{\bullet-}$ can be further transformed by manganese superoxide dismutase (Mn-SOD) to H_2O_2, or can react with nitric oxide (NO) to form peroxynitrite ($ONOO^-$). $O_2^{\bullet-}$ production leads to increased mitochondrial conductance through UCPs.

Citric acid cycle enzymes, oxidative phosphorylation enzymes and MAO are key mitochondrial enzymes studied in molecular psychiatry.

Citric acid cycle enzymes

Citrate synthase (EC 2.3.3.1, CS) catalyzes the condensation of acetyl CoA and oxalacetate to form citrate. It is the rate-limiting enzyme, controlling the flux of the TCA cycle, and plays a decisive role in regulating energy generation of mitochondrial respiration. Its activity is regulated by matrix phosphorylation potential, NAD^+/NADH ratio, and derivatives of fatty acids (Kispal et

al., 1989). It is used as quantitative marker for the presence of intact mitochondria (Scaini et al., 2010).

Succinate dehydrogenase (EC 1.3.99.1, SDH) is a reliable marker of the mitochondrial capability to supply an adequate amount of ATP (Scaini et al., 2010). SDH includes covalently attached flavin adenine dinucleotide (FAD) cofactor. In TCA cycle, it oxidizes succinate to fumarate along with reduction of FAD to $FADH_2$. In OXPHOS, electrons from $FADH_2$ are tunnelled and transferred to CoQ.

Oxidative phosphorylation enzymes

Complex I (EC 1.6.5.3, NADH:ubiquinone oxidoreductase, NADH dehydrogenase, NADH-ubiquinone oxidoreductase) is a crucial point of respiration. It catalyzes oxidation of reduced nicotinamide adenine dinucleotide (NADH), thus, regenerates NAD^+ for the TCA cycle and FAs oxidation, and reduces coenzyme Q_{10} (ubiquinone, CoQ) to ubiquinol (Hirst, 2009). Four protons are pumped from the matrix into the intermembrane space during electron passing through the complex I. Complex I is also a rate-limiting enzyme for oxygen consumption in the synapses (Telford et al., 2009).

Complex II (EC 1.3.5.1, succinate:ubiquinone oxidoreductase, succinate dehydrogenase (ubiquinone)) is the side entry into ETC, directly involved in the TCA cycle. It is a 4 subunit membrane-bound lipoprotein, which couples the oxidation of succinate to the reduction of CoQ (Tomitsuka et al., 2009). Complex II does not contribute to the proton gradient. Hence, complex II subunits are encoded only by nDNA, complex II is suspected to normalize the activity of ETC, when mtDNA defects are suspected (Saada et al., 2001).

Complex III (EC 1.10.2.2, ubiquinol:ferricytochrome-c oxidoreductase, CoQ-cytochrome *c* reductase) consists of two centers, Q_i center - facing to matrix; and Q_o center - oriented to intermembrane space (Chen et al., 2003). Complex III catalyzes the oxidation of one molecule of ubiquinol and the reduction of two molecules of cytochrome *c*. Reaction mechanism of complex III occurs in two steps called the Q cycle (Trumpower, 1990). In the process of Q cycle four protons are released into the inter membrane space.

Complex IV (EC 1.9.3.1, ferrocytochrome-*c*:oxygen oxidoreductase, cytochrome *c* oxidase, COX) enables the terminal reduction of O_2 to H_2O, retains all partially reduced intermediates

until full reduction is achieved (Turrens, 2003). The complex IV mediates pumping of 4 protons across the membrane. Previously, it was suggested as an endogenous metabolic marker for neuronal activity (Wong-Riley, 1989).

Complex V (EC 3.6.3.14, ATP synthase, F_oF_1-ATPase) consists of two regions: 1. F_1 portion is soluble domain with three nucleotide binding sites, it is localized above the inner side of the membrane and stably connected with F_o domain; 2. F_o portion is proton pore embedded in the membrane, it consists of three subunits and spans the membrane from the inner to the outer side (Noji and Yoshida, 2001; Zanotti et al., 2009). This formation enables the conversion of electrochemical potential energy to chemical energy - a portion of the F_o rotates as the protons pass through the membrane and forces F_1 as motor to synthetize ATP (Kadenbach, 2003; Noji and Yoshida, 2001).

Monoamine oxidase

Monoamine oxidase (MAO, EC 1.4.3.4) is located in the outer mitochondrial membrane and catalyses the oxidative deamination of amine neurotransmitters as well as xenobiotic amines. It regulates the metabolic degradation of catecholamines and serotonin (5-hydroxytryptamin, 5-HT) in neural and other target tissues. A major physiological role of intraneuronal MAO is to keep cytosolic monoamine concentrations very low. This membrane-bound enzyme is a flavoprotein, which use FAD as cofactor. The cofactor was identified as the site, where irreversible inhibitors of MAO are covalently linked (Youdim et al., 2006; Youdim and Bakhle, 2006). It exists in two isoforms MAO-A and MAO-B, they differ in substrate preference, inhibitory specificity, tissue and cell distribution, and in immunological properties (Bach et al., 1988). MAO-A metabolizes 5-HT and is sensitive to inhibition by low concentrations of clorgyline, whereas MAO-B prefers benzylamine or 2-phenylethylamine (PEA) as substrate and is sensitive to inhibition by low concentrations of l-deprenyl. Tyramine, tryptamine, dopamine, norepinephrine (NE) and epinephrine are equally well oxidized by both isoforms of MAO (Youdim et al., 2006). The high levels of both forms are found in the brain; MAO-B is found in dopamine-secreting neurons in the brain.

Monoamine metabolism by MAO involves oxidative deamination to corresponding aldehyde and free amine. Catalysis in MAO depends on the transfer of electrons to FAD, and mechanism-

based inhibitors, such as the irreversible antidepressants, modify flavin (Ramsay and Gravestock, 2003). The aldehyde is rapidly metabolized by aldehyde dehydrogenase to acidic metabolites. Metabolism of monoamines by MAO is a major source of hydrogen peroxide (H_2O_2) in the brain. Normally the H_2O_2 is then inactivated by glutathione peroxidase but it can be converted, chemically, by Fe^{2+} ions (Fenton reaction) into the highly reactive hydroxyl radical. This radical has widespread deleterious effects which can cause neuronal damage and death and may account for associated health-related problems (Youdim and Bakhle, 2006; Naoi et al., 2006).

MAOs have important role in brain development and function, and MAO inhibitors (MAOIs) have a range of potential therapeutic uses (Ramsay and Gravestock, 2003). Generally, selective inhibitors of MAO-A and nonselective MAOIs seem to be effective in the treatment of patients with depression, panic disorder, and other anxiety disorders (Stahl and Felker, 2008). It is supposed that MAO-B inhibition may slow the course of various neurodegenerative disorders; so, selective inhibitors of MAO-B may be efficacious in treating of Parkinson's disease (Horstink et al., 2006) and possibly Alzheimer's disease (Riederer et al., 2004). MAO-B is the sole type in human platelets and the amino acid sequences of MAO-B in both platelets and brain are identical (Chen et al., 1993); so, platelet MAO can be adopted as a useful surrogate model for the study of aspects of central neuronal function related to monoaminergic neurotransmission (Fišar and Raboch, 2008).

1.1.2 Regulation of OXPHOS

There are five levels of OXPHOS regulation: 1. direct modulation of ETC kinetic parameters, 2. regulation of intrinsic efficiency of OXPHOS (by changes in proton conductance, in the P/O ratio or in the channeling of ECT intermediate substrates), 3. mitochondrial network dynamics (fusion, fission, motility, membrane lipid composition, swelling), 4. mitochondrial biogenesis and degradation, 5. cellular and mitochondrial microenvironment (Benard et al., 2010).

OXPHOS efficiency is dependent on delivery of reducing equivalents into ETC and on activities of participating enzymes or enzyme complexes. The optimal efficiency and flow ratios are determined by control of complex I (reflects integrated cellular pathway) and complex II (TCA cycle precedes) (Cairns et al., 1998). Depletion of TCA cycle intermediates plays an important role in the OXPHOS flux control. In respirometry assays, supplies of complex I as well as complex II are required. Convergent electron input and reconstitution of the TCA cycle are

needed to achieve maximal respiration (Gnaiger, 2009). It is controlled also by the availability of adenosine 5´-diphosphate (ADP) for the adenine nucleotide transporter in the inner mitochondrial membrane (Ramzan et al., 2010).

Complex I is suggested to be responsible for adaptive changes and physiological set up of OXPHOS efficiency (Cocco et al., 2009). The stoichiometric efficiency of OXPHOS is defined by the P/O ratio, or the amount of inorganic phosphate (P_i) incorporated into ATP per amount of consumed oxygen. P/O ratio was analysed in rat brain, liver and heart mitochondria. There were found tissue-specific differences and dependency of the P/O ratio on the respiratory rates with complex I, but not with complex II substrates (Cocco et al., 2009). Metabolic control analysis, which compared ETC activities and oxygen consumption rates, determined the role of complex I in rat brain synaptosomes. Results of the study suggest complex I as rate-limiting for oxygen consumption and responsible for high level of control over mitochondrial bioenergetics (Telford et al., 2009).

As mentioned above, mitochondria exhibit transmembrane potential across the inner membrane that is necessary for OXPHOS. Protons are transported outwardly and create proton motive force (Δp), which consists of electrical part $\Delta\psi_m$ (negative inside) and chemical part ΔpH (Mitchell, 1961; Mitchell, 1966). In mitochondria, the Δp is made up of the $\Delta\psi_m$ mainly. The $\Delta\psi_m$ controls the ability of the mitochondria to generate ATP, generate ROS and sequester Ca^{2+} entering the cell. The $\Delta\psi_m$ and ATP synthesis express a degree of coupling; optimal ATP synthesis requires $\Delta\psi_m$ values between the range -100 mV and -150 mV. These values are reached primarily by $\Delta\psi_m$, which maintain at higher values (about -200 mV) and by secondary control mechanisms, which decrease the $\Delta\psi_m$ to lower levels (Kadenbach, 2003).Changes of $\Delta\psi_m$ influence permeability of biological membranes and ROS production, $\Delta\psi_m$ above -150 mV leads to exponentially increased permeability as well as $O_2^{\bullet-}$ and H_2O_2 production (Kadenbach et al., 2010). Similarly, mitochondrial membranes increase exponentially their permeability for protons (Kadenbach, 2003). On the other hand, lower mitochondrial Δp and $\Delta\psi_m$ (e.g. caused by inhibition of respiratory chain) can result in hydrolysis of cytoplasmic ATP and slightly lower potential than that generated by the respiratory chain (Nicholls et al., 2003). Therefore, $\Delta\psi_m$ is precisely controlled and can be regulated by various parameters.

ATP production is controlled by different mechanisms, depending on energy demands, thermogenesis, etc. (Kadenbach, 2003). First mechanism of OXPHOS control has been called as "respiratory control", and is based on feedback mechanisms controlling the rate of ATP synthesis, first of all by Δp and $\Delta\psi_m$. Higher levels of ADP in mitochondria lead to stimulation of ATP synthase together with decrease of Δp. Originally, pilot studies of OXPHOS dynamics used the terminology of respiratory steady states, described by Chance and Williams. Respiration was characterized by respiratory states (Table 1), by active state 3 (ADP stimulated) and followed by controlled state 4 (decrease after conversion of ADP to ATP) (Chance and Williams, 1956; Tager et al., 1983). Decreased P/O ratio (caused mostly by increased Δp) leads to energy waste - proton leak (slip in COX), the decrease in the coupling, and increased thermogenesis (Mourier et al., 2010). However, conception of states had limited applicability in intact cells and in isolated mitochondria, did not include for instance COX, adenine nucleotide transporter, and extramitochondrial ATP/ADP ratio.

Table 1 Characterization of respiratory states (Chance and Williams, 1955; Gnaiger, 2009)

	ADP level	***Substrate level***	***Respiration rate***	***Rate-limiting component***	***Relevance***
State 1	Low	Low-endogenous	Slow	Phosphate acceptor	Initial activity of the sample
State 2	High	Approaching zero	Slow	Substrate	1. Exhaustion of endogenous substrate utilized in OXPHOS of ADP 2. Residual oxygen consumption (ROX)
State 3	High	High	Fast	Respiratory chain	1. OXPHOS capacity at saturating ADP (State P) 2. Electron transfer system capacity at optimum uncoupler concentration (State 3u)
State 4	Low	High	Slow	Phosphate acceptor	1. Exhaustion of added ADP 2. LEAK respiration (resting state when oxygen flux is maintained mainly to compensate for the proton leak after inhibition of ATP synthesis) (States 4o, L)
State 5	High	High	Zero	Oxygen	1. Anoxia 2. Antimycin A treatment

Recently, primary control has been implemented by secondary control mechanisms that are Δp independent (Kadenbach, 2003; Walsh et al., 2009). Mitochondrial Ca^{2+} levels have been included. (Kadenbach et al., 2000). Ca^{2+} transport was presumed to be important only in buffering of cytosolic Ca^{2+} by acting as sink under conditions of Ca^{2+} overload. When the cytoplasmic Ca^{2+} level was overloaded, Ca^{2+} accumulated in mitochondrial matrix and utilized $\Delta\psi_m$ (McCormack et al., 1990; Rizzuto et al., 2000; Nicholls et al., 2003). Nowadays it is considered that Ca^{2+} regulates of activities of dehydrogenases via phosphorylation; ATP synthesis is switched on by cAMP-dependent phosphorylation and switched-off by calcium induced dephosphorylation (Lee et al., 2001; Ludwig et al., 2001).

In the TCA cycle, glycerophosphate dehydrogenase, pyruvate dehydrogenase, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase are influenced by Ca^{2+} levels and their phosphorylation lead to increased ATP production, production of glycogen, and glucose oxidation (Rizzuto et al., 2000). Reversible phosphorylation of pyruvate dehydrogenase complex mediated by calcium partly regulates the supply of reducing equivalents ($NADH/NAD^+$ ratio). Activation of the TCA cycle enhances the NADH production that triggers movement of electrons down complexes I through to complex IV by initially donating of complex I (Viola and Hool, 2010).

Regulation of complex I and COX subunits via specific protein kinases and protein phosphatases was observed. cAMP-dependent protein kinase catalyses phosphorylation of complex I subunit and stimulate the ETC (Papa et al., 2002). At low Ca^{2+} levels, protein phosphatase dephosphorylates and inactivates complex I. It is presumed that COX is regulated by allosteric inhibition of ATP at high ATP/ADP ratios (Kadenbach et al., 2010). Extramitochondrial ATP/ADP ratios regulate COX activity by binding to the cytosolic subunit of COX, whereas high mitochondrial ATP/ADP ratios cause exchange of ATP by ADP at COX and induce allosteric inhibition (Napiwotzki and Kadenbach, 1998). Similarly, increased intracellular Ca^{2+} levels are suggested to activate mitochondrial phosphatase, which dephosphorylates COX and turns off the allosteric inhibition (Lee et al., 2002). This respiratory control by phosphorylated enzyme is assumed to keep the Δp low as prevention of increased Δp, which leads to the slip of protons in COX and decreased H^+/e^- stoichiometry (Bender and Kadenbach, 2000; Arnold and Kadenbach, 1997). However, in isolated mitochondria high $\Delta\psi_m$ was measured even with high ATP/ADP ratios. The decrease was measured after addition of

phosphoenolpyruvate and pyruvate kinase and could be explained as reversal of gluconeogenetic enzymes (Ramzan et al., 2010). Under the physiological conditions, allosteric inhibition is modulated by increased Ca^{2+} levels, high substrate concentrations, and thyroid hormones. Ca^{2+} dependent dephosphorylation induced by hormones results in loss of respiratory control by the ATP/ADP ratio and associated with the increased Δp and respiration (Bender and Kadenbach, 2000).

Thyroid hormone, mainly triiodothyronine (T3) and diiodothyronine (T2), has important effects on mitochondrial energetics and mitochondrial genome (Cheng et al., 2010). Mechanism of allosteric inhibition of COX has been closely linked to regulation by thyroid hormones. 3,5-diiodothyronine (T2) mediates short term effects of thyroid hormones and increases immediately basal metabolic rate. T2 is formed by intracellular deiodination of T3 and binds to specific T2 binding sites, which were identified in the inner mitochondrial membrane (Goglia et al., 1999). This binding to subunit Va of COX abolishes the allosteric inhibition of respiration by ATP (Arnold et al., 1998) that could result in partial uncoupling of OXPHOS via increased $\Delta\psi_m$, and continue to intrinsic uncoupling of COX by higher membrane potentials (Kadenbach, 2003). Therefore, thyroid hormones enhance the proton permeability; hyperthyroidism stimulated mitochondrial proton leak and ATP turnover in rat hepatocytes, where non-mitochondrial oxygen consumption remained unchanged (Starkov, 1997; Harper and Brand, 1994). Oppositely, in rat hypothyroid cells significant decrease of non-mitochondrial oxygen consumption and proton leak were observed, ATP turnover was unaffected (Harper and Brand, 1993).

1.1.3 Proton permeability of membranes

OXPHOS in cells is not fully efficient. Decrease of the proton gradient across the inner mitochondrial membrane by “proton leak” causes uncoupling of fuel oxidation from ATP generation, and some energy is lost as heat. The mechanism of the basal proton conductance of mitochondria (insensitive to known activators and inhibitors) is not understood. There is correlation between mitochondrial proton conductance and composition of inner membrane: phospholipid fatty acyl polyunsaturation correlates positively and monounsaturation correlates negatively with proton conductance (Brand et al., 2003).

Uncoupling proteins (UCPs) and adenine nucleotide translocator (ANT) are two types of mitochondrial carrier, which cause inhibitor-sensitive inducible proton conductance. UCPs themselves do not contribute to the basal proton conductance of mitochondria; however, they are important metabolic regulators in permitting fat oxidation and in attenuating free radical production (Azzu et al., 2010). The amount of ANT present in the mitochondrial inner membrane strongly affects the basal proton conductance of the membrane and suggests that ANT is a major catalyst of the basal FA-independent proton leak in mitochondria (Brand et al., 2005).

Fatty acids

Long-chain fatty acids (FAs) are weak acids that can cross the membrane in both protonated and deprotonated forms. Effects of FAs are interrelated to 1. increase uncoupling, 2. increase ROS production, 3. opening mitochondrial permeability transition pores (MPTP) (Rial et al., 2010). Further, they can modulate effects of thyroid hormones as well as sex steroid hormones (Starkov, 1997). FAs can act as like classic OXPHOS uncouplers with protonophoric action on the inner mitochondrial membrane and/or interactions of FAs with ADP carrier, COX and ATP synthase are presumed (Wojtczak and Schönfeld, 1993). Recent study suggests that FAs are not only inducers of uncoupling, but they also regulate this process. It supposes that transport of FA anions participates in both ADP/ATP antiport and aspartate/glutamate antiport, at the same time (Samartsev et al., 2011). On the other hand, studies using lipid membranes suppose that FAs are capable of spontaneous flip-flop (Kleinfeld et al., 1997). Since FAs move across the membrane spontaneously and rapidly, no protein transporters are necessary. Further, coupling/uncoupling effects depend on their concentrations pH gradient across the membranes (Di Paola and Lorusso, 2006; Kamp and Hamilton, 1992).

Uncoupling proteins

Uncoupling diverts a significant proportion of energy to thermogenesis. UCPs are mitochondrial carriers catalysing a regulated proton leak across the inner membrane (Hagen and Vidal-Puig, 2002; Echtay et al., 2002). There are five types of UCP in mammals. UCP1 is presented exclusively in the inner mitochondrial membrane of brown adipose tissue, and its main

function is to catalyse adaptive thermogenesis (Brand et al., 2004). It can be stimulated by FA and has synergic action of norepinephrine and thyroid hormones (Zaninovich, 2005; Kadenbach, 2003). Concentrations of UCP2 and UCP3 in tissues are much lower than of UCP1, and their functions are not exactly known. They probably minimally contribute to basal metabolic rate, control of adaptive thermogenesis, preventive action against oxidative stress and ROS control, control of cellular energy balance, regulation of Ca^{2+} homeostasis, regulation of FA oxidation and ATP synthesis (Douette and Sluse, 2006; Boss et al., 2000; Trenker et al., 2007; De Marchi et al., 2011). UCP2, UCP4 and UCP5 are present in the central nervous system (CNS); they have been suggested to have effects protecting neurons from the Ca^{2+} overload and/or oxidative stress (Liu et al., 2006; Kwok et al., 2010).

UCP activities can be positively or negatively regulated by different factors. UCP are stimulated by FA and by ROS, generated by as a side reaction between CoQ and oxygen (Wolkow and Iser, 2006). UCP mediate the FA dependent proton influx that leads to uncoupled ATP synthesis and heat production (Beck et al., 2007). It is supposed that UCP and FA decrease $\Delta\psi_m$ if it is sufficiently high.

1.1.4 Reactive oxygen species production

Reduction of O_2 to water by aerobic respiration is accompanied by reactive intermediate formation. Generally, complex I and complex III are considered as the major $O_2^{\bullet-}$ sources (Ježek and Hlavatá, 2004).

Complex I releases $O_2^{\bullet-}$ to matrix, complex III can release $O_2^{\bullet-}$ to both sides of the inner mitochondrial membrane (Muller et al., 2004). Additionally, other ROS sources, e.g. MAO, present in the outer mitochondrial membrane, and α-ketoglutarate dehydrogenase (α-KGDH), the TCA cycle enzyme complex, are able to generate H_2O_2. MAO catalyses the oxidative deamination of biogenic and xenobiotic monoamines and increases the amount of ROS in mitochondria. H_2O_2 production by α-KGDH is dependent on NADH/NAD^+ ratio. Higher NADH leads to higher H_2O_2 production, therefore, α-KGDH could significantly contribute to oxidative stress in mitochondria (Tretter and Adam-Vizi, 2004).

Physiologically generated H_2O_2 and $O_2^{\bullet-}$ from ETC are dependent on the magnitude of Δp and the respiratory state of mitochondria (Murphy, 2009). State 4 is characterized with high rate of

ROS production, contrary to state 3 with high rate of oxygen uptake and slow ROS production. State 5, described as anoxic, with limited oxygen supply and lack of respiration produce minimum ROS (Brand et al., 2004; Cadenas and Davies, 2000). In isolated rat liver mitochondria ROS production and $\Delta\psi_m$ were studied in state 3 and state 4. These states attenuate $\Delta\psi_m$ and ROS, correlation of ROS with $\Delta\psi_m$ was observed (Tirosh et al., 2003). However, this correlation with respiratory states was not observed in the study using isolated mitochondria, ROS production correlated directly with $\Delta\psi_m$ (Aronis et al., 2003).

Complex I is considered to be the primary source of ROS in brain under physiological conditions, as well as in pathological processes (e.g. neurodegenerative disorders). ROS seem to be the key factors in brain aging processes and mitochondrial respiration with ROS production significantly contributes to functional changes in brain during aging. Study in isolated rat mitochondria found significantly increased H_2O_2 production and 30 % reduction of complex I activity in aged rats (Petrosillo et al., 2008). Defective mitochondria release large amounts of ROS, similarly, decline of antioxidative enzyme activities (e.g. in elderly) enhances ROS production (Schönfeld and Wojtczak, 2008). Negative results of ROS can affect respiratory chain: complexes I, III and IV seem to be the most affected, whereas function of complex II appears to be unchanged (Boffoli et al., 1994).

1.1.5 Apoptosis

Mitochondrial dysfunctions may accompany the clinical picture of neuropsychiatric disorders and contribute to neural apoptosis (Marazziti et al., 2012); mitochondria play a pivotal role in intrinsic pathway of apoptosis (Mattson et al., 2008). Several interrelated mitochondrial pathways facilitate cell death: mitochondrial permeability transition (MPT) and the release of apoptotic cell death promoting factors, cytochrome *c* release by proapoptotic members of the Bcl-2 (B-cell lymphoma 2) family of proteins, disruption of ATP production, and alteration of the cell's redox status and overproduction of ROS (Aronis et al., 2003). If they are activated, change their conformations and induce formation of oligomers to form mitochondrial outer membrane pores, resulting to MPT. In apoptotic cells rapid loss of mitochondrial $\Delta\psi_m$ is accompanied by ROS production. Consequently, other proapoptotic proteins cytochrome *c* and Smac are released and trigger the caspase cascade leading to apoptosis (Bolaños et al., 2009). Released cytochrome *c* in cytosol binds to apoptotic protease-activating factor-1 (Apaf-1) and induces formation of

apoptosome (Jiang and Wang, 2000). MPT means alteration of permeability properties of membranes, originally was defined as increase of the inner mitochondrial membrane permeability to solutes of molecular mass less than 1500 Da (Tsujimoto and Shimizu, 2007). Decreased MPT and activities of respiratory chain complexes, and increased ROS production were observed in cultured fibroblasts obtained from patients with CoQ deficiency (Rodríguez-Hernández et al., 2009). MPT results from formation and opening of a channel known as MPTP. MPTP is dynamic multiprotein complex that span both the outer and inner mitochondrial membrane and contain the adenine nucleotide translocator (ANT) in the inner membrane, and the voltage-dependent anion channels (VDAC) in the outer membrane and cyclophilin D in the matrix (Baines et al., 2007). Once open, MPTP allows the release of pro-apoptotic factors, such as cyt *c* and apoptosis inducing factor (AIF), into the cytoplasm.

1.1.6 Specific inhibitors of complexes of ETC

Rotenone is a specific complex I inhibitor, thenoyltrifluoroacetone (TTFA) specifically inhibits complex II. Both substances induce $O_2^{\bullet-}$ production that may result to major ROS production (Chen et al., 2007; Turrens, 2003; Foster et al., 2006). Pyrrolnitrin inhibits both complex I as well as complex II. It affects electron transport among NADH, CoQ and succinate, whereas COX remains unaffected (Wong et al., 1971).

Complex III inhibitors antimycin, myxothiazol and stigmatellin differ in their mechanism of action. Antimycin A inhibits the transfer of electrons from cytochrome *b* to CoQ, blocks the Q_i side of complex III. Oppositely, myxothiazol or stigmatellin block electron transfer from reduced CoQ at Q_o side (Viola and Hool, 2010). Stigmatellin inhibits transfer of electrons and recycling of CoQ; myxothiazol inhibits electron transfer from reduced CoQ to cytochrome *c* (Raha et al., 2000).

Complex IV inhibitors KCN and sodium azide decrease COX activity (Ferguson et al., 2005). Azide specifically blocks crossover between cytochrome *a* and cytochrome a_3. Further, it inhibits succinate oxidase activity specific for active respiration (state 3), but without any significant inhibition of state 4 (Wilson and Chance, 1967). Inhibition of COX by KCN is reversible, cyanide inhibits both electron and proton transport of COX (Nůsková et al., 2010). Complex V is inhibited by oligomycin by blocking its proton channel (F_o subunit). This inhibitor increases $\Delta\psi_m$

and is used to prevent state 3 of respiration. Oligomycin induces artificially state 4, i.e. state of respiration independent of ADP phosphorylation or resting state (LEAK) (Sjövall et al., 2010).

During the oxidation of complex I substrates (pyruvate, malate, glutamate), rotenone inhibition did not increase H_2O_2; contrary, oxidation of complex I and II substrates in the presence of antimycin A increases H_2O_2. Both myxothiazol and stigmatellin inhibited $O_2^{\bullet-}$ production and/or should inhibit the effect of antimycin (Konstantinov et al., 1987; Raha et al., 2000). The maximum of $O_2^{\bullet-}$ production has been observed in human skin fibroblasts with the prolonged treatment of rotenone, but not with antimycin A (Koopman et al., 2010). Interestingly, rotenone prevented antimycin A to induce ROS production in complex I, but not in complex II (Chen et al., 2003). Q_o side of complex III was found as the source of increased $O_2^{\bullet-}$ after transient exposure to hydrogen peroxide (Viola and Hool, 2010). KCN and sodium azide increase ROS formation (Ferguson et al., 2005). Oligomycin induces hyperpolarization of inner mitochondrial membrane and can increase $O_2^{\bullet-}$ levels (Kirkland and Franklin, 2007).

1.1.7 Mitochondria and neuroplasticity

Mitochondrial distribution and activity are key factors in neuronal morphogenesis - synaptogenesis, developmental and synaptic plasticity and axogenesis. During the development, neuronal stem cells proliferate and differentiate into neurons; subsequently axons and dendrites form synapses (Mattson and Partin, 1999; Erecinska et al., 2004). The role of mitochondria in neuroplasticity is illustrated in Fig. 3 (Hroudová and Fišar, 2011). Due to ATP production and importance of mitochondria in synaptic ion homeostasis and phosphorylation reactions, mitochondria would be accumulated at sites where ATP consumption and Ca^{2+} concentration are higher. It was reported that mitochondria are more abundant in the regions of growing axons than in the non-growing axons. Mitochondrial net movement is anterograde in growing axons and is retrograde in non-growing axons. Shortly before axogenesis mitochondria congregate at the base of the neurite that is destined to become the axon. Nerve growth factor (NGF) was found as one of the signals inducing accumulation of mitochondria in the active growing cone (Chada and Hollenberck, 2004). Interestingly, when the ATP production is impaired and cells provide alternative source of energy, axogenesis is abolished although growth of dendrites remains relatively unaffected (Mattson and Partin, 1999).

There are changes in mitochondrial energy metabolism occurring in brain cells during CNS development. During embryonic and early postnatal development fats are primarily used, later on, glucose becomes as fuel. This fact supports the role of mitochondria in biochemical requirements of highly proliferative neuronal stem cells and post-mitotic neurons. During neuronal differentiation the number of mitochondria per cell increases, but the velocity at which individual mitochondria move decreases as neurite outgrowth slows and synaptogenesis occurs (Chang and Reynolds, 2006; Hroudová and Fišar 2011).

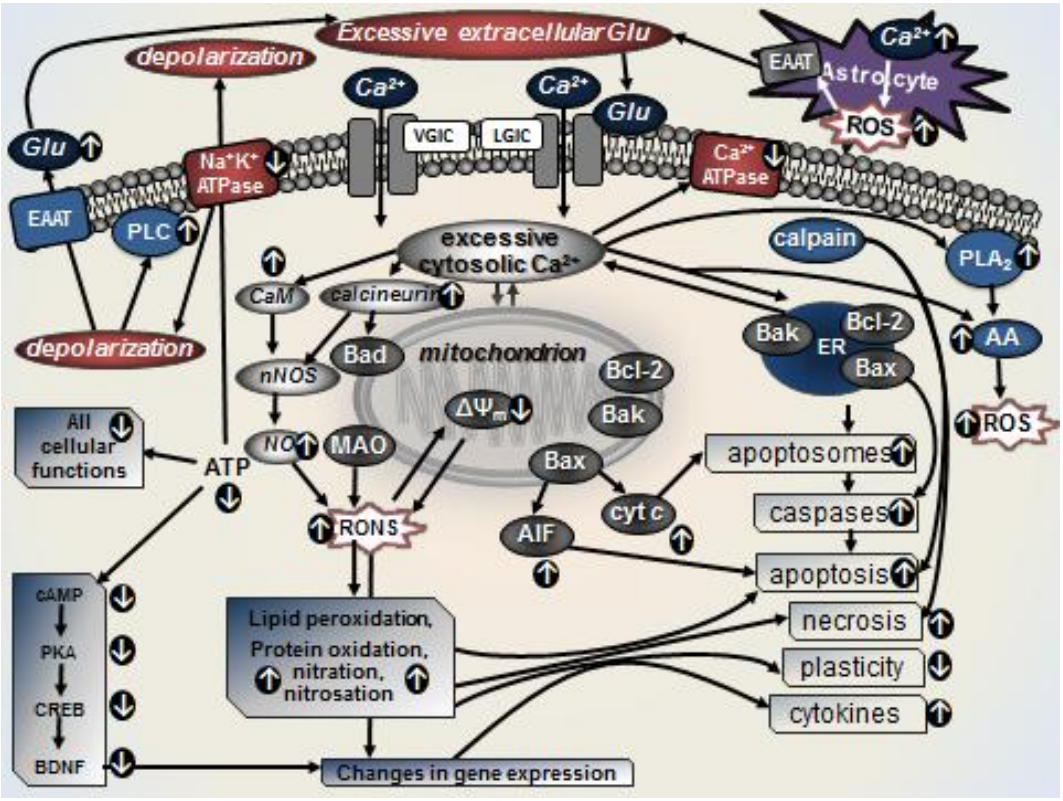


Fig. 3 The role of mitochondria in neuroplasticity (Hroudová and Fišar, 2011)**.** Principal mechanisms leading to neuronal impairment and cell death are composed of decreased ATP production, increased production of reactive oxygen and nitrogen species (RONS), initiation of apoptotic processes and impaired calcium homeostasis. Exhaustion of energy supplies and decreased ATP production lead to impairment of ATP dependent processes and therefore to changed cellular functions. Insufficient function of Na^+/K^+- ATPases leads to disturbances of ion transmembrane gradients, efflux of K^+, and influx of Na^+, Cl^- and Ca^{2+}. Increased extracellular concentrations of K^+ mediate depolarisation of membranes and change the functions of amino acids transporters. Voltage gated ion channels (VGIC) and ligand dependent calcium channels (LGIC) are activated and mediate increased cytosolic calcium concentrations. Intracellular calcium causes functional changes of amino acid transporters and enhances the increased extracellular concentrations of EAA, glutamate especially, and extends neurotoxicity. Increased levels of synaptic glutamate can be mediated by release of glutamate from astrocytes. Following bound of glutamate to NMDA and AMPA receptors causes higher Ca^{2+}influx into cell, calcium activates phospholipases, proteases, and endonucleases, which degrade membranes, proteins and nucleic acid. E.g. activation of phospholipase A_2 (PLA_2) by calcium releases membrane arachidonic acid (AA), which induces production of superoxide. High intracellular

calcium levels cause overload of mitochondrial calcium, increase ROS production, and inhibit ATP production. Activation of calcium dependent protein phosphatases (e.g. calcineurin) causes translocation of proapoptotic factor Bad into the mitochondria and triggers apoptosis by sequestration of antiapoptotic factors Bcl-2 and Bcl-xL. Release of cytochrome c and other proapoptotic factors from the intermembrane space of mitochondria induce the formation of apoptosome, and consequently trigger activation of caspases and apoptosis. Apoptosis inducing factor (AIF) is another factor released by mitochondria. Disengaged AIF is transported into nucleus and trigger caspases-independent apoptosis. Mitochondria in brain are also a target of nitric oxide (NO) action.

AA - arachidonic acid; AIF - apoptosis inducing factor; Bax, Bad, Bad - proapoptotic factors of Bcl-2 family; Bcl-2 - antiapoptotic factor of Bcl-2 family; BDNF - brain-derived neurotrophic factor; CaM - calmoduline; cAMP - cyclic adenosine monophosphate; CREB - cAMP response element-binding protein; cyt *c* - cytochrome *c*; $\Delta\psi_m$ - potential on the inner mitochondrial membrane; EAAT - excitatory amino acid transporter; ER - endoplasmic reticulum; Glu - glutamate; MAO - monoaminoxidase; nNOS - neuronal nitric oxide synthase; NO - nitric oxide; PKA - protein kinase A; PLA_2 - phospholipase A_2; PLC - phospholipase C; LGIC - ligand-gated ion channel; RONS, reactive oxygen and nitrogen species; ROS - reactive oxygen species; RNS - reactive nitrogen species; VGIC - voltage-gated ion channel

It was demonstrated that neuronal activity is influenced by the mitochondrial functions, defective trafficking and dysfunction of mitochondria from axon terminals is implicated in the pathogenesis of axonal degeneration (Cai and Sheng, 2009; Stamer et al., 2002; Konradi et al., 2004). In addition, dendritic mitochondria are essential in the morphogenesis and plasticity of spines and synapses (Overly et al., 1996). Recent findings suggest roles for mitochondria as mediators of at least some effects on glutamate and BDNF on synaptic plasticity (Chada and Hollenberck, 2004). BDNF promotes synaptic plasticity, in part, by enhancing mitochondrial energy production. It increases glucose utilization and increases mitochondrial respiratory coupling at complex (Cocco et al., 2009; Markham et al., 2004).

Mitochondria are dynamic organelles; their function is modulated by fission, fusion and moving within the axons and dendrites (Mattson et al., 2008). Their structure, functions and properties differ in axons and dendrites (Overly et al., 1996; Ruthel and Hollenberck, 2003). Transport and positioning of mitochondria is essential for neuronal homeostasis and the mitochondrial movement is a part of regulation by intracellular signals.

1.2 Advances in biological hypotheses of mood disorders

Findings about intracellular processes associated with mood disorders and long-term effects of antidepressants demonstrate an important role of signalling pathways primarily regulated by monoamine neurotransmitters; this was settled as the basis of many biochemical hypotheses (Fišar, 1998; Fišar et al., 2009). While dysfunctions within monoaminergic neurotransmitter

systems are likely to play an important role in pathophysiology of mood disorders, it probably represents the downstream effects of more primary abnormalities in signal transduction. Thus, new theories about the pathophysiology of depression and the action of antidepressant treatment proposes that mood disorders are caused by structural or functional changes in particular molecules and signalling pathways in the brain, and that antidepressants function by counteracting these molecular changes. It is supposed that structural and functional brain abnormalities in patients with depressive disorder may be associated with low levels of brain-derived neurotrophic factor (BDNF), abnormal function of hypothalamic-pituitary-adrenal (HPA) axis, glutamatergic toxicity, activation of inflammatory and cell-mediated immune response, decreased antioxidant capacity and increased oxidative and nitrosative stress, disturbed chronobiological rythms, and mitochondrial dysfunctions (Krishnan and Nestler, 2008; Mathew et al., 2008; aan het Rot et al., 2009; Maes et al., 2012).

Research on the biological basis of mood disorders emphasises the changes of neural networks and synaptic plasticity. Evidence exists for impairment of neuroplasticity in major depression. Chronic stress is known to contribute both to development of major depression in vulnerable persons and to reduction of synaptic plasticity, induction of structural changes in dendrites, and impairment of neurogenesis (Pittenger and Duman, 2008). Mitochondria may be primary regulators of these processes, as they regulate not only neuronal survival and death, but also plasticity. There is mounting evidence for the role of mitochondrial dysfunction in the pathophysiology and treatment of bipolar disorder (Quiroz et al., 2008).

1.2.1 Monoamine hypothesis

Discovery of the first effective antidepressants, MAOIs and tricyclic antidepressants, implied hypothesis about significant role for the biogenic amine, particularly NE and 5-HT in the ethiopathogenesis of affective disorders. Classic monoamine hypothesis is an early milestone in the field of depression. It proposed that depression might be produced by a 5-HT or NE deficiency at functionally important receptor sites in the brain, i.e. that brain monoamine systems have a primary direct role in depression (Coppen, 1967; Schildkraut, 1965). Soon it became evident that the monoamine hypothesis in its original form could not explain all of the effects of antidepressants (Nestler et al., 2002; Hindmarch, 2002). In order to test this hypothesis, a series of studies was conducted to evaluate effects of monoamine depletion on depressive symptoms in

depressed patients and in healthy controls. Relapse to 5-HT depletion or to catecholamine depletion was found to be specific to the type of antidepressant treatment and type of depletion. 5-HT or NE/dopamine depletion did not decrease mood in healthy controls and slightly lowered mood in healthy controls with a family history of major depressive disorder. In drug-free patients with major depressive disorder in remission, a moderate mood decrease was found for acute tryptophan depletion only. However, acute tryptophan depletion induced relapse in patients in remission who used serotonergic antidepressants (Delgado et al., 1999). Depletion studies failed to demonstrate a causal relation between 5-HT and NE with depressive disorder (Ruhé et al., 2007; Cowen, 2008). The effects of acute tryptophan depletion on cognition in non-vulnerable participants are independent of mood changes (Mendelsohn et al., 2009). Even simultaneous disruption of 5-HT and catecholamine systems didn't significantly alter mood in unmedicated depressed subjects (Berman et al., 2002). These findings forced a major revision of the classic monoamine hypothesis of depression. According to this revised monoamine theory of depression (Heninger et al., 1996; aan het Rot et al., 2009) monoamine systems are only modulating other brain neurobiological systems that have more primary role in depression.

1.2.2 Neurotrophic hypothesis

The neurotrophic hypothesis of depression (Duman et al., 1997; Duman, 2002; Zarate et al., 2006; Einat and Manji, 2006) supposed that vulnerability to depression can arise as a result of neuronal damage, e.g. after chronic stress, long-term increased levels of glucocorticoids, hypoglycemia, ischemia, effects of neurotoxins or certain viral infections, etc. The therapeutic effects of antidepressants consist in the increased function of the noradrenergic or serotonergic system, leading to increased activity of transcription factor CREB (cAMP response element binding protein), higher expression of neurotrophin BDNF and its receptor trkB, and consequently to increased neuronal plasticity and resumption of cellular functions.

According to neurogenic hypothesis (Jacobs, 2002; Jacobs et al., 2000), depression may develop due to the decreased neurogenesis in hippocampus, and antidepressants takes effect through the stimulation of neurogenesis. Hypothesis of cellular plasticity (Kempermann and Kronenberg, 2003) relate the neurotrophic and the neurogenic hypothesis to the statement that depression can be generally caused by damaged cellular plasticity leading to inadequate relations

between structure and function. Molecular mechanisms leading to a disturbance of neuroplasticity are not known. The bioenergetic and neurochemical model of bipolar disorder attempts to identify these mechanisms and focuses attention on mitochondrial dysfunctions (Kato and Kato, 2000; Stork and Renshaw, 2005).

1.2.3 Inflammatory and neurodegenerative hypothesis

The central nervous system, endocrine and immune systems use neurotransmitters, cytokines and hormones to communicate among them (Haddad et al., 2002). Now there is evidence that the activation of the immune system is associated with the symptoms of depression (Leonard and Myint, 2009; Catena-Dell'Osso et al., 2011). The inflammatory and neurodegenerative hypothesis of depression (Maes et al., 2009) supposes that depression is associated with both inflammatory processes, as well as with neurodegeneration and reduced neurogenesis. According to this hypothesis, enhanced neurodegeneration and impaired neurogenesis in depression are caused by inflammatory processes, related to the production of oxidative and nitrosative stress, tryptophan catabolites along the indoleamine-2,3-dioxygenase pathway, proinflammatory cytokines and lowered ω-3 polyunsaturated fatty acid status. Anti-inflammatory compounds should be able to counteract at least partly the enhanced neurodegeneration and decreased neurogenesis.

1.2.4 Mitochondrial hypothesis

Mitochondrial dysfunctions (leading to decreased ATP production, oxidative stress, and induction of apoptosis) occur in the early stages of different neurodegenerative diseases, associated often with mood disorders.

The role of mitochondrial dysfunction during bipolar disorder is supported both by observation of the changes of brain metabolism and by effects of mood stabilizers (lithium and valproate) on mitochondrial functions. Metabolic changes in brain were observed in bipolar disorder by magnetic resonance spectroscopy (MRS). It suggests the presumptions that mitochondrial dysfunctions include impaired OXPHOS, final shift to glycolytic production of

energy, general decrease of energy (decreased ATP production), changed concentrations of phosphomonoesters and changed lipid metabolism (Stork and Renshaw, 2005).

mtDNA mutations in the brain, associations of mtDNA polymorphisms and bipolar disorder and changes in gene expression related to mitochondria in the brain were observed (Kato, 2008; Iwamoto et al., 2005). Mitochondrial dysfunction hypothesis of bipolar disorder is based on these observations. According to this hypothesis, mtDNA polymorphisms/mutations or mtRNA deletions caused by nuclear gene mutations can cause mitochondrial dysregulation of calcium leading to symptoms of bipolar disorder (Kato and Kato, 2000; Kato, 2007; Kato, 2008). Mitochondrial hypothesis corresponds to, above mentioned, neurotrophic hypothesis because of an important role of calcium signalling pathway in synaptic plasticity regulation.

1.2.5 Biological markers of mood disoders

Biological markers are defined as a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention. In medicine, a biomarker is an indicator of a particular disease state or a particular state of an organism.

Identification of biologic markers of mood disorders and factors capable of predicting the response to treatment with antidepressants has not been sufficiently successful (Balon, 1989; Joyce and Paykel, 1989; Fišar and Raboch, 2008). In accordance to actual neurochemical hypotheses of mood disorders, biological markers have been primarily found at the level of neurotransmitter concentrations, their metabolites or precursors. Subsequently, attention was shifted to the receptor systems, and since the 1990´s, intracellular processes have become main interest. The chance to find sensitive and specific biological predictors of antidepressant treatment has been increased, because of introduction of new methods of molecular biology. These methods enable us better observation of cellular processes connected with the transduction of nervous signals in the brain. The choice of parameters, which should be studied as perspective biological markers of mood disorders, have been derived first of all from new findings of signalling pathways involved in neurotransmission and from above mentioned neurochemical hypotheses of mood disorders. From the view of intracellular processes, energetic metabolism, activities of PKC, CREB, BDNF, Bcl-2, glycogen synthase kinase-3, caspases or calcium could play a principal role in findings of biological markers of mood disorders. According to the

complexity and connectivity of signalling pathways involved in etiopathogenesis of mood disorders, number of chosen parameters is not final.

1.3 Antidepressants, mood stabilizers and mitochondrial functions

Antidepressants are used mainly to alleviate mood disorders, such as major depression and dysthymia and anxiety disorders. Mood stabilizers are psychiatric medication used in treatment of mood disorders, which are characterized by intense and sustained mood shifts (e.g. bipolar disorder).

The antidepressant activity of the first generation of antidepressants, tricyclic antidepressants and MAOIs, was explained by their effects on availability of monoamine neurotransmitters. The next generations of antidepressants included selective serotonin reuptake inhibitors (SSRIs), norepinephrine reuptake inhibitors (NRIs), serotonin-norepinephrine reuptake inhibitors (SNRI), noradrenergic and specific serotonergic antidepressants (NaSSAs), norepinephrine-dopamine reuptake inhibitors (NDRIs), serotonin antagonist and reuptake inhibitors (SARIs), selective serotonin reuptake enhancer (SSRE), melatonergic agonists (MASSA), sigma receptor agonists etc. The therapeutic response to antidepressants occurs after long-term treatment; therefore, effects of antidepressants are linked to cellular adaptations including density and/or sensitivity of neurotransmitter receptors and transporters, regulation of signal transduction cascades, and changes in gene expression (Duman, 2004).

Most of mood stabilizers are anticonvulsants (valproate, carbamazepine, and lamotrigine), with an important exception of lithium, which is the oldest and the best known mood stabilizing drug. Some atypical antipsychotics (olanzapine, quetiapine, aripiprazole, risperidone, ziprasidone) have mood stabilizing effects, as well.

Although a wide range of pharmacologically different antidepressants and mood stabilizers is available, molecular mechanisms of their therapeutic effects haven’t yet been sufficiently clarified. Relatively little information is known about the association among therapeutic and/or adverse effects of drugs and mitochondrial enzyme activities. Incomplete data exist on the effect of pharmacologically selective antidepressants and mood stabilizers on MAO activity. Measurement of both mitochondrial respiration and membrane potential during action of appropriate endogenous and exogenous substances enables the identification of the primary sites

of effectors and the distribution of control, allowing deeper quantitative analyses (Brand and Nichols, 2011).

1.3.1 Inhibition of MAO

MAO inhibition is the best known direct action of some antidepressants on mitochondrial enzymes. The antidepressant effect of MAOIs has been established more than 50 years ago. Iproniazid became the first MAO inhibitor to be used successfully in the treatment of depression; it is an irreversible and nonselective MAO inhibitor (Fagervall and Ross, 1986). It is known to act as a pro-drug and can be converted into isopropyl hydrazine which binds covalently to MAO (Smith et al., 1963). Clorgyline is an irreversible inhibitor preferential for MAO-A, structurally related to pargyline (MAO-B inhibitor). It has antidepressant activity, and may potentially be useful in the treatment of Parkinson's disease. Selegiline (l-deprenyl) is an irreversible inhibitor preferential for MAO-B; it is used for the treatment of Parkinson's disease, depression and senile dementia. Inhibitors of MAO lose its selectivity at high doses. Moreover, there are feedbacks and interconnections of intracellular signalling pathways which lead to mutual interactions of monoaminergic and other systems (Fišar and Hroudová, 2010b; Fišar 2012). So, inhibiting of MAO-B should influence processes mediated primarily by substrates for MAO-A, and vice versa. The major disadvantage was the incidence of the cheese reaction with those early inhibitors (Youdim and Bakhle, 2006).

The selective reversible MAO-A inhibitors such as moclobemide increase the content of 5-HT, NE and dopamine in the brain (Haefely et al., 1992) but did not provoke the cheese reaction. Moclobemide has been extensively evaluated in the treatment of a wide spectrum of depressive disorders and social phobia. Overall, moclobemide appears to be safe and devoid of major side effects, although it is considered as a mild antidepressant, better tolerated by older patients (Lecrubier, 1990; Lecrubier and Guelfi, 1994; Iwersen and Schmoldt, 1996; Lotufo-Neto et al., 1999; Madger et al., 2000; Bonnet, 2003; Praško et al., 2006). Moclobemide undergoes extensive metabolism with less than 1 % of the dose being excreted unchanged. Metabolic pathways of moclobemide include mainly oxidative attack on the morpholine moiety (Jauch et al., 1990). However, major metabolites in plasma were found to be less effective MAO-A inhibitors than moclobemide or pharmacologically inactive (Da Prada et al., 1989; Baker et al., 1999).

MAO inhibitors were developed as antidepressants but many drugs, including the oxazolidinone antibacterial agents, share similar molecular properties and have MAO inhibitory activity. These compounds were of interest as potential antidepressants because they could be selective inhibitors of either the A or B isoforms and were usually reversible (Ramsay and Gravestock, 2003).

Antidepressants which act primarily as 5-HT and/or NE reuptake inhibitors show inhibitory activity towards MAO also. It has been suggested that tricyclic antidepressants exert some of their therapeutic effect by inhibiting MAO (Reyes and Lisansky, 1984). They are able to inhibit MAO-B both *in vitro* (Roth and Gillis, 1974; Edwards and Burns, 1974) and *in vivo* (Sullivan et al., 1977; Sullivan et al., 1978). However, *in vivo* inhibition of the human platelet MAO-B in the patients taking tricyclic antidepressants was not confirmed by others (Davidson et al., 1978; Reveley et al., 1979). Five tricyclic antidepressants, amitriptyline, clomipramine, desipramine, imipramine and iprindole, have comparable potencies as inhibitors of MAO in rodent brain and liver (Green and McGachy, 1987). These antidepressants have been shown to partially protect mouse brain MAO *in vivo* from the irreversible enzyme inhibition produced by subsequent injection of phenelzine (Green et al., 1989). Concentrations of tricyclic antidepressants, which showed a pronounced inhibitory effect on the MAOs activity, were significantly higher than plasma levels of the drug found under therapeutic conditions (Honecker et al. 1976; Nag, 2004). MAO activity was inhibited after long-term administration of viloxazine, nomifensine, zimelidine, maprotiline, imipramine, amitriptyline, and nortriptyline in systematic studies of Egashira (Egashira et al., 1996; Egashira et al., 1999). Competitive inhibition of MAO-A and noncompetitive inhibition of MAO-B was found for these drugs. Similar results were obtained when different tricyclic antidepressants and SSRIs were examined with isolated rat brain mitochondria (Gnerre et al., 2001). Fluoxetine and norfluoxetine showed affinities both for MAO-A (Mukherjee and Yang, 1999) and MAO-B (Mukherjee and Yang, 1997). Fluoxetine and norfluoxetine also significantly inhibited the binding of the specific radioligands to MAO *in vivo*. These results support a potential role of MAO inhibition in the therapeutic effects of fluoxetine.

1.3.2 Effects of antidepressants on mitochondrial functions

There is relatively little data about effects of antidepressants on mitochondrial functions as summarized in the Table 2. *In vitro* study examined influence of pharmacologically different antidepressants and mood stabilizers on activity both mitochondrial MAO (Fišar et al., 2010) and respiratory chain complexes; imipramine, desipramine, amitriptyline, citalopram, and mirtazapine were found as complex I inhibitors in isolated pig brain mitochondria (Hroudova and Fisar, 2010). In isolated rat liver mitochondria effects of imipramine and clomipramine were compared to classic uncouplers, drugs enhanced ATP synthase activity, hindered ATP synthesis and released respiratory control (Weinbach et al., 1986). In isolated rat liver mitochondria, nefazodone was found as inhibitor of mitochondrial complexes I and IV; buspirone inhibited complex I but had no effect on complex IV. Trazodone did not effect on both complex I and complex IV (Dykens et al., 2008), but decreased oxygen consumption and reduced Na^+, K^+-ATPase activity. Trazodone acts also as uncoupler of OXPHOS (Velasco et al., 1985).

Effects of antidepressants on apoptotic markers, e.g. cytochrome *c* release and DNA fragmentation, seem to be different. Various antidepressants exhibited potential anticancer properties and caused cytotoxic effects. Paroxetine, fluoxetine and clomipramine increased levels of apoptotic markers leading to apoptosis in glioma and neuroblastoma cells, whereas imipramine and mianserin do not (Levkovitz et al., 2005). Desipramine induced apoptosis in rat glioma cells by activation of caspases, without any change of mitochondrial membrane potential $\Delta\psi_m$ (Ma et al., 2010). Fluoxetine and amitriptyline protected PC12 cells from cell death induced by hydrogen peroxide (Kolla et al., 2005). Amitriptyline and tranylcypromine prevented the loss of mitochondrial $\Delta\psi_m$, over expression of Bax, reduction in Bcl-2 level, cytochrome *c* release, caspase-3 activation, and formation of ROS. In contrast, fluoxetine seemed to have additive toxic effect to 1-methyl-4-phenylpyridinium (MPP^+) against neuronal cell damage by increasing mitochondrial damage and oxidative stress (Han and Lee, 2009). Nortriptyline was identified as strong inhibitor of MPT and was observed as potential inhibitor of neuronal cell death; it protected isolated mitochondria against programmed cell death, inhibited release of apoptotic mitochondrial factors and caspases, increased Ca^{2+} retention in mitochondria and delayed the Ca^{2+} induced loss of $\Delta\psi_m$, further leading to neuronal cell death (Wang et al., 2007; Zhang et al., 2008).

Table 2 Effects of antidepressants on mitochondrial functions

Antidepressant	Biological model	Affected mitochondrial function	Reference
Imipramine	Isolated rat liver mitochondria	Uncoupling effects on OXPHOS (release of respiratory control, hindered ATP synthesis, enhanced ATP synthase activity)	Weinbach et al., 1986
	Beef heart submitochondrial particles	Inhibition NADH oxidation, inhibition of ATP synthase	
Imipramine	Rat brain mitochondria	Increased state 3 and state 4 respiratory rates	Katyare and Rajan, 1995
Imipramine	Rat liver mitochondria	Increased state 3 and state 4 respiratory rates	Katyare and Rajan, 1988
Imipramine, clomipramine, citalopram	Human peripheral lymphocytes and lymphoblasts	Dose-dependent induction of apoptosis	Xia et al., 1997 Karlsson et al., 1998
Imipramine, clomipramine, citalopram	Human acute myeloid leukaemia HL-60 cells	Loss in cell viability, increased ROS production, loss of $\Delta\psi_m$	Xia et al., 1999
Clomipramine, desipramine, norfluoxetine,	Rat heart isolated mitochondria	Reductions of $\Delta\psi_m$, Decrease in state 3 respiration	Abdel-Razaq et al., 2012
	CHO cells	Inhibition of activities of complexes I, II/III and IV	
Tianeptine	Rat heart isolated mitochondria	Insignificant change of $\Delta\psi_m$, decrease in state 3 respiration	
	CHO cells	Inhibition of complex I activity	
Tianeptine	Rat liver mitochondria	Inhibited beta-oxidation and TCA cycle	Fromenty et al., 1989
Fluoxetine	Rat liver mitochondria	Inhibition of state 3 respiration, stimulation of state 4 respiration, decrease of RCR and uncoupling effects on OXPHOS	Souza et al., 1994
Fluoxetine	Rat brain mitochondria	Inhibition of OXPHOS, decreased activity of ATP synthase	Curti et al., 1999
Amitriptyline, fluoxetine	Differentiated rat pheocytochroma PC12 cells	Prevention of the loss of $\Delta\psi_m$, cyt *c* release, formation of ROS induced by MPP^+	Han and Lee, 2009

Amitriptyline, fluoxetine	Rat pheocytochroma cells	Attenuation of H_2O_2 neurotoxic effects, upregulation of superoxide dismutase	Kolla et al., 2005
Nortriptyline	ALS mouse*	Strong inhibitor of MPT	Wang et al., 2007
Nortriptyline	Mouse model of ischemia	Inhibition of $\Delta\psi_m$, inhibited release of mitochondrial factors and caspase 3 activation	Zhang et al., 2008
Nortriptyline	Rat brain mitochondria	Inhibitor of MPT, inhibition of ETC, mild uncoupling	Morota et al., 2009
Fluoxetine and/or olanzapine	Rat brain homogenates	Increased CS activity after acute, but not chronic treatment	Agostinho et al., 2011
Nefazodone Trazodone	Isolated rat liver mitochondria	Severe inhibition of oxygen consumption Inhibition complexes I and IV Modest inhibition of oxygen consumption Inhibition of complex I	Dykens et al., 2008
Nefazodone Trazodone	Isolated rat liver mitochondria	Complex I and complex IV inhibitor No effects	Velasco et al., 1985
Fluoxetine	Hippocampal synaptic plasma membranes	Increased ATP synthase activity	Gamaro et al., 2003
Sertraline	Isolated rat liver mitochondria	Uncoupling effects on OXPHOS, inhibition of complex I and complex V activities, induction of Ca^{2+} mediated MPT	Li et al., 2012
Venlafaxine, paroxetine, nortriptiline	Rat brain homogenates (after 15 days of drug administration)	Differences in brain areas: increased or unchanged CS and SDH activities	Scaini et al., 2010
Paroxetine, fluoxetine, klomipramine	Rat glioma and human neuroblastoma cell lines	Increased cyt *c* release, caspase-3-like activity, induction of apoptosis	Levkovitz et al., 2005
Desipramine	Rat glioma cells	Activation of caspases 3 and 9, no changes of $\Delta\psi_m$	Ma et al., 2011

*ALS mouse – model of neurodegeneration

1.3.3Effects of mood stabilizers on mitochondrial functions

Mood stabilizers affect multiple sites in intracellular signalling pathways (Fišar and Hroudová, 2010b). Main targets of mood stabilizers are neurotrophin BDNF, ERK pathway, and pathways modulated by GSK-3 or Bcl-2 (Einat and Manji, 2006; Gould and Manji, 2005; Shaltiel et al., 2007). Molecular and cellular targets of mood stabilizers include enzymes inhibited by lithium (inositol monophosphatase, inositol polyphosphate 1-phosphatase, GSK-3, fructose 1,6-bisphosphatase, bisphosphate nucleotidase, phosphoglucomutase), enzymes inhibited by valproate (succinate semialdehyde dehydrogenase, succinate semialdehyde reductase, histone deacetylase), targets of carbamazepine (sodium channels, adenosine receptors, adenylate cyclase), and components of signalling pathways regulated by multiple drugs (PKC, cAMP, arachidonic acid) (Gould et al., 2004). Furthermore, lithium and valproate reduce transport of myo-inositol into the cells, which leads to reduced PKC activity. Lithium and valproate increase Bcl-2 concentrations (Chen et al., 1999) and inhibit GSK-3 activity (lithium directly, valproate indirectly). Valproate activates MAPK signalling pathway and regulates stress proteins of ER (Bown et al., 2002). Through the effects on Bcl-2 and p53 (proapoptotic protein), lithium affects mitochondria by stabilization of membrane integrity and prevention of MPTPs opening; i.e. by regulating the key process in cell death leading to at least temporary loss of $\Delta\psi_m$, input of water into matrix and equilibration of ions concentrations. Both lithium and valproate have neuroprotective effects based on protection from glutamatergic neurotoxicity by inactivation of NMDA receptors, on activation of cell survival factors such as phosphoinositide 3-kinase/protein kinase B pathway, and on induction of neurotrophic and neuroprotective proteins. Lithium protects against DNA damage, caspases activation, and apoptosis of neurons (Chuang, 2005). Increased concentrations of N-acetyl aspartate (NAA, marker of neuronal viability and functionality) in grey matter after the chronic lithium administration support its strong neuroprotective and neurotrophic effects in humans.

Effects of mood stabilizers on monoaminergic activity have been studied; majority of data is about the effects of lithium. Lithium enhances the antidepressant effect both of MAOIs and inhibitors of the reuptake of 5-HT and/or NE (Magder et al., 2000; Nierenberg et al., 2006; Kitaichi et al., 2006). The mode of action for the lithium augmentation of antidepressants is partly mediated by an increase of 5-HT neurotransmission (Redrobe and Bourin, 1999; Haddjeri

et al., 2000; Muraki et al., 2001). However, lithium could not either inhibit MAO-A or MAO-B in the brain mitochondrial (Nag, 2004; Fišar et al., 2010). Unipolar and bipolar depressive patients showed significantly higher platelet MAO activity than controls, but there was no significant change in activity after the institution of lithium treatment (Reveley et al., 1981).

Studies have shown effects of mood stabilizing drugs on mitochondria. In isolated brain mitochondria lithium caused desensitisation to calcium, antagonized permeability transition, and diminished cytochrome *c* release (Shalbuyeva et al., 2007). In isolated rat liver mitochondria valproate inhibited OXPHOS (Haas et al., 1981).In isolated pig brain mitochondria both lithium and valproate inhibited respiratory chain complexes I and IV (Hroudova and Fisar, 2010). According to study performed in rats (Corrêa et al., 2007), valproate reversed the decreased activity of CS caused by amphetamine and lithium prevented the inhibition. The cytoprotective effect of lithium and valproate was observed after 7 days, of pre-treatment of human neuroblastoma (SH-SY5Y) cells against cytotoxicity resulting from oxidative stress evoked by rotenone and H_2O_2. This effect was not observed after one day of pre-treatment (Lai et al., 2006). Chronic treatment of SH-SY5Y cells prevents reduction of methamphetamine-induced reduction of cytochrome *c*, mitochondrial antiapoptotic Bcl-2/Bax ratio and mitochondrial COX activity (Bachmann et al., 2009). Interestingly, long-term lithium and valproate did not protect SH-SY5Y cells against endoplasmic reticulum stress-induced cytotoxicity (Lai et al., 2006). Lithium and carbamazepine could facilitate activation of CREB, valproate and lamotrigine did not affect BDNF-mediated signalling (Mai et al., 2002). Thus, these mood stabilizers likely decrease the vulnerability of mitochondrial functions caused by oxidative stress and have neuroprotective effects (Lai et al., 2006).

Chronic treatment with lithium, valproate and carbamazepine protects against NMDA-mediated toxicity (Bown et al., 2003). Interestingly, recent study performed with epileptic children examined the influence of carbamazepine and lamotrigine on mitochondrial functions - both drugs influenced respiratory chain complexes and significantly affected ATP production, carbamazepine decreased the production, oppositely to stimulatory effect of lamotrigine (Berger et al., 2010). Carbamazepine interferes in adenylate cyclase pathway: inhibits adenylate cyclase and the synthesis of cAMP (Chen et al., 1996). Lamotrigine prevented the toxicity caused by rotenone and MPP^+ in rat PC12 cells by suppressing the MPT formation, which leads to cytochrome *c* release and subsequent apoptosis. Though, lamotrigine seems to have

neuroprotective effect due to the mitochondrial respiratory complex I inhibition (Kim et al., 2007).

Effects of mood stabilizers on mitochondrial functions are summarized in the Table 3.

Table 3 Effects of mood stabilizers on mitochondrial functions

Mood stabilizer	Biological model	Affected mitochondrial function	Reference
Valproate	Rat liver mitochondrial fractions	Inhibition of the rate of oxygen consumption, sequestration of intramitochondrial CoA	Ponchaut et al., 1992
Valproate	Isolated rat liver mitochondria	State 3 rates of oxygen consumption inhibited	Haas et al., 1981
Valproate	Isolated beef brain α-KGDH	Inactivation of α-KGDH complex	Luder et al., 1990
Valproic acid	Isolated rat hepatocytes	CoA, acetyl-CoA and long chain acyl-CoA fractions decreased (accumulation of valproyl-CoA; without any evidence of this metabolite in brain tissue)	Becker and Harris, 1983
Valproate and its metabolites	Submitochondrial particles prepared from rat liver	Inhibition of pyruvate uptake	Aires et al., 2008
Valproate	Rat liver mitochondria Digitonin permeabilized rat hepatocytes	Inhibition of pyruvate-driven OXPHOS Inhibition of the rate of ATP synthesis (pyruvate as substrate used, no inhibitory effects caused by succinate and glutamate as substrates)	Silva et al., 1997
Valproate and lithium	Rat brain tissue obtained from animals pretreated by d-amphetamine	No modification of complex I, II, III and IV activities after the treatment with valproate and lithium in controls	Valvassori et al., 2010
Valproate and lithium	Rat brain tissue obtained from animals pretreated by d-amphetamine	Treated animals with lithium and valproate prevented inhibition caused by d-amphetamine	Corrêa et al., 2007
Valproate and lithium	Rat brain tissue obtained from animals pretreated by d-amphetamine	Treated animals with lithium and valproate reversed ATP synthase activity (increased after d-amphetamine)	Zugno et al., 2009

Valproate and lithium	Human neuroblastoma and glioma cells	Protective effects against H_2O_2 or rotenone induced cytotoxicity in neuroblastoma cells	Lai et al., 2006
Valproate and lithium	Human neuroblastoma cells	Reduction of methamphetamine-induced reduction of cytochrome *c*, antiapoptotic Bcl-2/Bax ratio and COX activity	Bachmann et al., 2009
Lithium	Plasma synaptic membrane from rat brain (submitted to animal model of depression)	Impaired function of ATP synthase was modulated (reversed by lithium treatment, prevented by lithium pretreatment)	de Vasconcellos et al., 2005
Lithium	Isolated brain mitochondria	Desensitisation to calcium, antagonized MPT, diminished cytochrome *c* release	Shalbuyeva et al., 2007
Lithium	Postmortem human brain cortex	Dose-depedent increased activities of complexes I+III, II+III and succinate dehydrogenase	Maurer et al., 2009
Lithium	Human neuroblastoma SH-SY5Y cells	Attenuation of rotenone-induced caspase-3 activation	King et al., 2001
Carbamazepine	Rat liver mitochondria	Decreased state 3 respiration, RCR, ATP synthesis, $\Delta\psi_m$	Santos et al., 2008
Carbamazepine	Rat brain mitochondria	Protection against rotenone induced complex I inhibition	Costa et al., 2008
Carbamazepine, lamotrigine	Human white blood cells	Carbamazepine decreased ATP production, stimulatory effect on production by lamotrigine	Berger et al., 2010
Lamotrigine	Human neuroblastoma SH-SY5Y cells	Suppression of MPT formation, attenuation of rotenone-toxicity, inhibition of ROS production	Kim et al., 2007

1.3.4 Metabolic changes in mood disorders

According to Kato (Kato et al., 2001; Kato and Kato, 2000; Kato, 2008) mtDNA polymorphisms/mutations or mtDNA deletions caused by mutations of nuclear genes could cause mitochondrial dysregulation of calcium, leading to the symptoms of bipolar affective disorder. Mitochondrial changes then alter energy metabolism, change concentration of

phosphomonoesters and production of lipids. Abnormal cellular energy and phospholipid metabolism was found using proton (^{1}H) and phosphorus (^{31}P)-MRS (Stork and Renshaw, 2005). Lower creatine and phosphocreatine as well as choline-containing compounds (glycerophosphocholine and phosphocholine) were found in postmortem studies of left dorsolateral prefrontal cortex in medication-free patients suffering from bipolar disorder (Frey et al., 2007a; Frey et al., 2007b). MRS studies demonstrated also increased lactate, decreased pH, phosphocreatine, and ATP levels; shift toward to glycolytic energy production. Studies using two-dimensional proton echo-planar spectroscopic imaging (PEPSI) confirmed altered brain metabolism in medication-free patients suffering from bipolar disorder. Patients exhibited increased grey matter lactate and γ-aminobutyric acid (GABA) levels; phosphocreatine, creatine or choline-containing compounds were not altered (Dager et al., 2004). Postmortem studies of patients with bipolar disorder have identified anatomical and neuropathological abnormalities including ventricular enlargement, decreased gray matter volume, and reductions of number, size and/or density of neurons and glial cells (Rajkowska, 2002; Strakowski et al., 2005; Vawter et al., 2006). Analyses using PET and single-photon emission computed tomography (SPECT) demonstrate variable loss of monoamines in depressive patients. PET analyses of brain energy metabolism suggested mitochondrial deficits in idiopathic psychiatric disorders. Data demonstrated reduced ATP production rate and increased mitochondrial deletions in patients compared to controls. Patients suffering from depression exhibit reduced glucose utilization in the prefrontal cortex, anterior cingulated gyrus and caudate nucleus (Videbech, 2000).

2. Hypothesis and aim of the study

Molecular psychiatry applies for the study of biological basis of mental and neurodegenerative disorders interdisciplinary approaches, especially neurobiological, neurochemical, neurophysiological, neuroimmune, neuroendocrine and genetic approach. New biological hypotheses of mental disorders have been formulated on the basis of interconnections and feedback effects of signalling pathways participating on the nervous signal transmission. Principal sources of knowledge come from structural and functional changes in the brain, effects of stress, chronobiology, activity of hypothalamus-pituitary-adrenal axis, changes of immune functions, synthesis and metabolism of neurotransmitters, density or affinity of their receptors and mediators, and first of all changes in intracellular signaling pathways down streaming on neurotransmitters systems.

Mood disorders are probably related to disturbed signal transduction through the chemical synapse resulting from organic, physiological as well as environmental stimuli, included in etiology of mood disorders. It is supposed that impaired bioenergetics of neurons participate on pathophysiology of the disorder. We presume that therapeutic effects of psychopharmaca, presently administered or newly developed for the treatment of depression, can be find 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 complex response to stress, neurotoxicity or impaired neurotransmission.

On the basis of this hypothesis, we study effects of depressive disorder, antidepressants and other psychoactive drugs with pharmacologically different mechanisms of action on activities of mitochondrial enzymes. It includes isoforms of MAO, enzymes of TCA cycle and complexes of ETC providing OXPHOS and ATP production as the main source of energy for metabolism, synthesis and active transport within the cells.

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 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 diagnosis and treatment as well as in the development of new drugs with the specific effects on these newly discovered intracellular targets. Thus, the primary aim of this

study was discovery of the drug-induces changes in selected mitochondrial functions using antidepressants, mood stabilizers and animal brain mitochondria as *in vitro* model. Subsequent aim was application of these findings into the preclinical research of mitochondrial dysfunctions in blood platelets of patients suffering from depression.

We applied radiochemical, spectrophotometrical, fluorescence methods and high-resolution respirometry. Effects of pharmacologically different antidepressants and mood stabilizers on activity of mitochondrial enzymes (MAO-A, MAO-B, citrate synthase, complexes of ETC) and on mitochondrial respiration were measured using pig brain mitochondria as *in vitro* model. Effects of depressive disorder on activity of mitochondrial enzymes (MAO-B, citrate synthase, complexes of ETC) and on mitochondrial respiration were measured using human platelets.

3. Materials and methods

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 based on the facts that the bioenergetics function of mitochondria can be investigated by measurement of rate of ATP formation and efficiency of the process (the P/O ratio, i.e. the ratio of ATP formed over oxygen consumed) (Merlo-Pich et al., 2004) and that measurement of oxygen consumption and its sensitivity to substrates, uncouplers and inhibitors can be good indication of mitochondrial phosphorylation capacity.

To provide a routine approach to the study of oxygen kinetics, multiple substrate-uncoupler-inhibitor titration (SUIT) protocols for high-resolution respirometry were developed for accurate measurement with small amounts of tissue, cells and isolated mitochondria (Pesta and Gnaiger, 2012). Measurement rests mainly on 1. instrumental design to achieve minimum oxygen diffusion into a homogenously stirred closed chamber, 2. polarographic oxygen sensors and electronics providing sufficient stability and resolution of dissolved oxygen in a dynamic range of 10^4, 3. high time resolution of the signal, 4. software-supported signal correction based on standardized instrumental calibrations, and 5. resolution of non-linear changes of rate based on the time derivative of oxygen concentration (Gnaiger, 2001).

Isolated mitochondria from animal brains (Whittaker, 1969) serve as proper *in vitro* model for study of mitochondrial functions. Mitochondrial functions in disease have been often investigated in muscle biopsies (Fischer et al., 1985; Pesta and Gnaiger, 2012). Blood platelets represent a system that possess mitochondria and may be easily separated from human blood. Moreover, platelets are used as a good model for neurons in some biological parameters (Da Prada et al., 1988) and in study of mitochondrial dysfunctions in neurodegenerative disorders (Schapira, 1998) or in aging (Lenaz et al., 2002). Thus, intact and permeabilized cells, and permeabilized muscle fibers are used for functional mitochondrial diagnosis with high-resolution respirometry.

3.1 Animal brain mitochondria

Fresh pig brains were obtained on slaughter-house and rapidly placed to ice-cold buffered sucrose (0.32 mol/l sucrose, 4 mmol/l HEPES; pH 7.4). All subsequent procedures were

performed at 0-4 °C. Brain cortex was separated without cerebellum, gently homogenized in ten volumes (w/v) of ice-cold 0.32 mol/l buffered sucrose supplemented with aprotinin (competitive serine protease inhibitor), by means of a homogenizer with Teflon piston. Mitochondria were prepared by a standard differential centrifugation method (Whittaker, 1969). Briefly, the homogenate was centrifuged at 1000 g for 10 min to remove unbroken cells, nuclei and cell debris. The supernatant was carefully decanted; the pellet was resuspended in buffered sucrose and centrifuged again under the same conditions. Supernatants were collected and recentrifuged at 10000 g for 15 min. The final pellet containing crude mitochondria was washed twice with buffered sucrose (10000 g, 15 min), 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 enzymes of citric acid cycle and respiratory chain complexes

3.2.1 Preparation of mitochondria for enzyme assays

Crude mitochondrial fraction was resuspended with hypotonic buffer (25 mmol/l potassium phosphate, 5 mmol/l $MgCl_2$, pH 7.2), and suspension was frozen and thaw two times to achieve the maximum of enzyme activities (Kirby et al., 2007). Samples were incubated with selected psychopharmaca for 30 minutes at 30 ºC. Final drug concentrations were 5 mmol/l for lithium and valproate, 500 μmol/l for desipramine, amitriptyline, imipramine, citalopram, venlafaxine, mirtazapine, tianeptine, moclobemide, and olanzapine. Samples were measured at 30 ºC and in a total reaction volume of 3 ml; final protein concentration was 150 μg/ml. Activities of respiratory chain complexes and enzymes of citric acid cycle were measured spectrophotometrically using Uvicon XL spectrophotometer (SECOMAM, Alès, France). All chemicals were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Enzyme assays used in our study are stated below.

3.2.2 Enzyme assays

Complex I (NADH dehydrogenase (ubiquinone), EC 1.6.5.3)

NADH dehydrogenase activity was measured as the rotenone-sensitive rate of NADH oxidation at 340 nm. Previously published method was used (Ragan et al., 1987; Folbergrová et al., 2007) with a slight modification. The reaction medium was composed of 25 mmol/l potassium phosphate (pH 7.2), 5 mmol/l $MgCl_2$, 2.5 mg/ml bovine serum albumin (BSA), 2 mmol/l KCN, and 0.3 mmol/l NADH. The reaction was started by the addition of coenzyme Q_1 (in final concentration 33 µmol/l) and measured for 10 min. Afterwards rotenone was added in final concentration 50 µmol/l and the inhibited rate was measured for further 2 min.

Complex II (succinate dehydrogenase (ubiquinone), EC 1.3.5.1)

The activity of succinate dehydrogenase complex was measured as a decrease of absorbance of 2, 6-dichlorophenolindophenol (DCPIP, artificial acceptor of electrons) for 3 minutes at 610 nm. The reaction mixture contained 25 mmol/l potassium phosphate buffer (pH 7.2), 5 mmol/l $MgCl_2$, 20 mmol/l sodium succinate, 50 µmol/l DCPIP, 2 mmol/l KCN, 2 µmol/l antimycin A, and 2 µmol/l rotenone. The reaction was initiated by the addition of coenzyme Q_{11} to final concentration 60 µmol/l (Trounce et al., 1996).

Complex IV (cytochrome-c oxidase, EC 1.9.3.1)

Cytochrome-*c* oxidase activity was measured as a decrease of absorbance during oxidation of reduced cytochrome *c* at 550 nm. The reaction mixture was consisted of 20 mmol/l KH_2PO_4 (pH 7.0), 0.45 mmol/l lauryl maltoside; reaction was started with reduced cytochrome c (final concentration 25 µmol/l) and was monitored for 1 min (Rustin et al., 1994).

Succinate dehydrogenase (EC, 1.3.99.1)

A slight modification of the method of Munujos et al. (1993) was used for the determination of succinate dehydrogenase activity. Incubation mixture was composed of: 100 mmol/l Tris/HCl, pH 8.3, 0.5 mmol/l EDTA, 2 mmol/l KCN, 0.1% Triton and 20 mmol/l potassium succinate adjusted at pH 7.4. After the incubation, reaction was initiated with addition of 0.3% iodotetrazonium chloride - final concentration 2 mmol/l) and was continuously measured for 10 minutes at 500 nm.

Citrate synthase (CS 2.3.3.1)

The activity of CS was measured as a color change of 5, 5´-dithiobis-(2-nitrobenzoic) acid (DNTB). Incubation medium was composed of 100 mmol/l Tris/HCl (pH 8.1), 0.1% Triton X-100, 0.2 mmol/l DTNB, and 0.3 mmol/l acetyl-CoA. The reaction was initiated by the addition of 0.5 mmol/l oxaloacetate and absorbance was measured at 412 nm for 3 min (Srere et al., 1969).

Data analysis and statistics

Enzyme activities were evaluated as a slope of time dependence of absorbance of samples using LabPower Junior software (SECOMAM). Each independent measurement had a control, i.e. sample containing all components except for the drug. Relative changes of enzyme activities evoked by drugs were determined assuming that the activity of the control sample is equal to 100 %. Residual enzyme activity, i.e. activity at very high drug concentration, was determined in our inhibitory experiments. The full inhibitory curve was measured only for the effect of desipramine on complex I activity. This inhibition was analyzed using the four-parameter logistic function (SigmaPlot, Systat Software. Inc., Richmond, CA, USA), to establish the half maximal inhibitory concentration (IC_{50}), residual activity and Hill slope (coefficient).

All data presented are expressed as the mean ± standard deviation. Results were analyzed by STATISTICA (data analysis software system, version 9.0, StatSoft, Inc., Tulsa, OK, USA). The Wilcoxon matched pairs test (a nonparametric alternative to the t-test for dependent samples) was used to calculate test statistics in order to compare the enzyme activities in samples with the drug and without the drug.

3.3 Drug effects on monoamine oxidase activity

MAOs activity was determined radiochemically (Ekstedt, 1976; Egashira et al., 1978; Ozaita et al., 1997; Egashira et al., 1999) using either [^{14}C]5-HT (maximum concentration 100 μmol/l) or [^{14}C]PEA (maximum concentration 10 μmol/l) as substrates, respectively. At such concentrations these amines have been shown to behave as specific substrates for the A and B isoforms of MAO, respectively (Fowler and Tipton, 1981; Youdim et al., 2006).

Mitochondria were diluted in modified Krebs-Henseleit buffer without Ca^{2+} (KH solution; 118 mmol/l NaCl, 4.7 mmol/l KCl, 1.2 mmol/l KH_2PO_4, 1.2 mmol/l $MgSO_4$, 25 mmol/l

$NaHCO_3$, 11.1 mmol/l glucose; pH 7.4). The reaction mixture containing the crude MAO and various drug concentrations was preincubated for 60 min at 37 °C. Reaction was started by addition of radiolabelled substrate and MAO activity was measured at 37 °C for 30 min for MAO-A and for 1 min for MAO-B.

MAO-A activity was measured using [^{14}C]5-HT solution with specific activity of approximately 40 kBq/ml which was prepared by mixing of [^{14}C]5-HT stock solution (5-hydroxytryptamine binoxalate 5-[2-^{14}C], specific activity of 1.85 GBq/mmol, radioactive concentration 3.7 MBq/ml, radiochemical purity greater than 99 %, American Radiolabeled Chemicals, Inc., St. Louis, MO, USA) with KH buffer and unlabelled (cold) serotonin (5-hydroxytryptamine hydrochloride, Sigma-Aldrich Co., St. Louis, MO, USA).

MAO-B activity was measured using [^{14}C]PEA solution with specific activity of approximately 40 kBq/ml which was prepared by mixing of [^{14}C]PEA stock solution (2-phenylethylamine [ethyl-1-^{14}C] hydrochloride, specific activity of 2.03 GBq/mmol, radioactive concentration 3.7 MBq/ml, radiochemical purity greater than 99 %; American Radiolabeled Chemicals) with unlabeled PEA (2-phenylethylamine hydrochloride; Sigma-Aldrich Co.) solution in KH buffer.

The final sample volume was 250 µl containing 200 µg of protein. The reaction was stopped by the addition of 250 µl of 2 N hydrochloric acid. The reaction products, i.e. the corresponding aldehydes of 5-HT and PEA after oxidative deamination, were extracted into benzene:ethyl acetate 1:1 (v/v) and the radioactivity of the extracts in the organic phase were measured by liquid scintillation counting (LS6000IC, Beckman Instruments, Inc., Fullerton, CA, USA). Both MAO assays were performed over times where product formation was shown to proceed linearly under the condition used so that the values obtained corresponded to the initial velocities of the enzyme-catalyzed reaction.

Since some drugs are poorly soluble in aqueous media, they were sonicated in KH solution to obtain homogenous suspension. Iproniazid was used in the form of phosphate salt; clorgyline and pargyline were used in the form of hydrochlorides (all purchased from Sigma-Aldrich Co.).

Inhibition of MAO-A and MAO-B activities

Dependence of MAO activity on drug concentration was measured at different final concentrations (at least 7 concentrations). The effect was examined of desipramine, amitriptyline, imipramine, fluoxetine, citalopram, venlafaxine, reboxetine, mirtazapine, tianeptine, moclobemide, lithium, valproate, olanzapine, iproniazid, pargyline, and clorgyline. Blank values were obtained by addition of the hydrochloric acid before the substrate was added. MAO-A activity was measured using 16 μmol/l [^{14}C]5-HT solution. The final [^{14}C]5-HT concentration in samples was 3.2 μmol/l. MAO-B activity was measured using 50 μmol/l [^{14}C]PEA solution. The final [^{14}C]PEA concentration in samples was 10 μmol/l. The remaining MAO-A and MAO-B activities were expressed as portions of control basal activity and plotted as a function of the drug concentration. The reversibility of the inhibition of MAO by drugs was proven using the dilution method (Ulus et al., 2000).

Determination of enzyme kinetic parameters

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. Kinetic constants for MAO-A were assessed with seven different concentrations of [^{14}C] serotonin (5, 8, 10, 12.5, 25, 50, 100 μmol/l) using aliquots of 250 μmol/l [^{14}C]5-HT solution. Blank values were obtained by the addition of 100 μmol/l clorgyline before the substrate was added. Similarly, kinetic constants for MAO-B were determined with seven different concentrations of [^{14}C]PEA (0.5, 0.8, 1, 1.25, 2.5, 5, 10 μmol/l) using aliquots of 25 μmol/l [^{14}C]PEA solution. Blank values were obtained by the addition of 1 μmol/l pargyline before the substrate was added.

Data analysis

Inhibition of MAO activity by drugs was analyzed using the four-parameter logistic function (SigmaPlot, Systat Software. Inc., Richmond, CA, USA), to establish the half maximal inhibitory concentration (IC_{50}) and Hill slope (coefficient). Hill slope characterizes the slope of the curve at its midpoint and it is used in determining the degree of cooperativity of the ligand binding to the enzyme. IC_{50} represents the concentration of a drug that is required for 50% inhibition of

enzymatic reaction at a specific substrate concentration. IC_{50} values are dependent on conditions under which they are measured; so, data obtained are valid for existing enzyme concentration and type of inhibition. Because inhibitory potency is related to the dissociation constant of the enzyme-inhibitor complex (K_i, the reciprocal of the binding affinity of the inhibitor to the enzyme) this parameter can be a predictor of *in vivo* inhibitory potency. The analysis shown that K_i is equal to IC_{50} under conditions of either non-competitive or uncompetitive kinetics; however, K_i does not equal IC_{50} when competitive inhibition kinetics applies (Cheng and Prusoff, 1973). IC_{50} and K_i can be related for competitive reversible inhibitor of monosubstrate reaction by Cheng-Prusoff equation:

$$K_i = IC_{50}/(1+S/K_m),$$

where S is substrate concentration.

The MAO activity obeyed simple Michaelis-Menten kinetics with maximum rate V_{max}, and K_m (numerically equal to the substrate concentration required at rate of $V_{max}/2$). Different effects of the inhibitor on V_{max} and K_m result from its binding to the enzyme, to the enzyme-substrate complex, or to both. Reversible enzyme inhibitors can be classified as competitive, noncompetitive, uncompetitive, or mixed. We used nonlinear regression (SigmaPlot with Enzyme Kinetics Module) to calculate the parameters V_{max}, K_m, and to determine the mechanisms of inhibition. Akaike's information criterion (Akaike, 1974) with a second order correction for small sample sizes was used as a tool for model selection, i.e. as a measure of the goodness of fit of an estimated type of inhibition.

3.4 Drug effects on mitochondrial respiratory rate

Materials

Mitochondrial respiration medium (MiR05) consisted of sucrose 110 mmol/L, K-lactobionate 60 mmol/L, taurine 20 mmol/L, $MgCl_2.6H_2O$ 3 mmol/L, KH_2PO_4 10 mmol/L, EGTA 0.5 mmol/L, BSA 1g/L, HEPES 20 mmol/L, adjusted to pH 7.1 with KOH (Kuznetsov et al., 2004; Pesta and Gnaiger, 2012). Substrates, inhibitors or drugs were added to samples containing mitochondria as described in protocols below. Hamilton syringes were used for manual titration, automatic titration-injection micropump TIP2k (Oroboros Instruments, Innsbruck, Austria) was used for drug titration. Effects of various antidepressants (amitriptyline, fluoxetine, tianeptine),

mood stabilizers (lithium carbonate, sodium valproate, olanzapine), and ketamine were tested. Effect of chlorpromazine was measured for comparison. Chemicals were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA), except for fluoxetine, tianeptine and olanzapine, which were gifts from Zentiva Group a.s. (Prague, Czech Republic).

High-resolution respirometry

Activity of mitochondrial respiratory system was measured at 37 °C in titration-injection high-resolution oxygraph (Oxygraph-2k, Oroboros Instruments, Innsbruck, Austria) equipped with two closeable tempered chambers with Clark-type polarographic oxygen electrodes. The sample was placed into incubation chamber and stirred continuously using magnetic stirring bar. High-resolution respirometry is based on monitoring of oxygen concentration in the sample over time and calculation of rate of oxygen consumption.

Data were collected and analyzed using software DatLab 4.3 (Oroboros Instruments, Innsbruck, Austria) displaying the real-time oxygen concentration and oxygen flux, which is the negative time derivative of oxygen concentration. Oxygen solubility factor relative to distilled water for MiR05 was set to 0.92 (Sjövall et al., 2010). Respiratory rates (oxygen fluxes) were expressed as pmol O_2 consumed per second relative to one milligram of protein in the sample of crude brain mitochondria (pmol/mg.s).

Drug effects on respiration rate of brain mitochondria

Isolated pig brain mitochondria were used as an *in vitro* model to study the effects of antidepressants and mood stabilizers on mitochondrial oxygen consumption rate. Digitonin was used for permeabilization of the plasma membrane of synaptosomes presented in crude mitochondrial fraction. Digitonin permeabilizes the plasma membranes, whereas mitochondrial membranes are affected only at higher concentrations. To differentiate effect of drugs on the respiratory capacities attainable through complex I and complex II, separate respirometric measurements for complex I as well as complex II were performed. Thus, two protocols were used for determination of changes in respiration rate induced by different concentrations of amitriptyline, fluoxetine, tianeptine, ketamine, lithium, valproate, olanzapine, and chlorpromazine. At least 17-point titration was performed, thus final drug concentrations were in the range 0.5 to 2500 μmol/L. Because one titration experiment takes more than 1 hour and respiratory rate of sample in closed chamber significantly decrease during this period, two

parallel samples were titrated and measured simultaneously, sample in the first chamber was titrated by the drug and the sample in the second was titrated by medium used for dissolving of the drug.

2.0 mL MiR05 in measuring chambers were saturated with atmospheric oxygen for about 30 min, chambers were closed and 2-10 µL crude mitochondria was injected so that final protein concentration in the sample was achieved between 50 and 200 µg/mL;10 µL digitonin (50 µg/mL) was added immediately to permeabilize synaptosomes.

a) Respiration through complex I was initiated by adding 2 mmol/L malate and 5 mmol/L pyruvate. Following signal stabilization, the state 3_{CI} respiration was induced by the addition of 1.25 mmol/L ADP and titration with 0.5 – 100 µL drug/medium was carried out using TIP2k with 3 min interval between single titrations. Finally, the complex I was blocked by 0.5 µmol/L rotenone and residual oxygen consumption (*ROX*) was measured to be subtracted from all other values of respiratory rate.

b) Activation of respiration through complex II was preceded by addition of 1.25 mmol/L ADP and by complex I inhibition by 0.5 µmol/L rotenone. Then the state 3_{CII} respiration was induced by the addition of 10 mmol/L succinate and titration with 0.5 – 100 µL drug/medium was carried out using TIP2k with 3 min interval between single titrations. Finally, respiration was blocked by 1.25 µg/mL antimycin A, which inhibits the transfer of electrons from cytochrome *b* to CoQ, blocks the Q_i side of complex III. Residual oxygen consumption (*ROX*) was measured to be subtracted from all other values of respiratory rate.

Following *ROX* subtraction from respiratory rates at different drug/medium concentrations, differences among drug-titrated and medium-titrated samples were calculated and relative drug-induced changes in respiratory rate were determined, supposing that relative respiratory rate equals to 1 at zero drug concentration.

Data analysis

All data presented are expressed as the mean ± standard deviation. Results were analyzed by STATISTICA (data analysis software system, version 10.0, StatSoft, Inc., Tulsa, OK, USA). The differences in means between two groups were evaluated with Mann-Whitney *U* test. Inhibition of respiratory rate by drugs was analyzed using the four-parameter logistic function (SigmaPlot, Systat Software Inc., Richmond, CA, USA), to establish the concentration of a drug that is

required for 50% inhibition *in vitro* (IC_{50}) and Hill slope, which characterizes the slope of the inhibition curve at its midpoint and it is used to determine the degree of cooperativity of the ligand binding to the enzyme.

Typical record of the measurement is represented in Fig. 4.

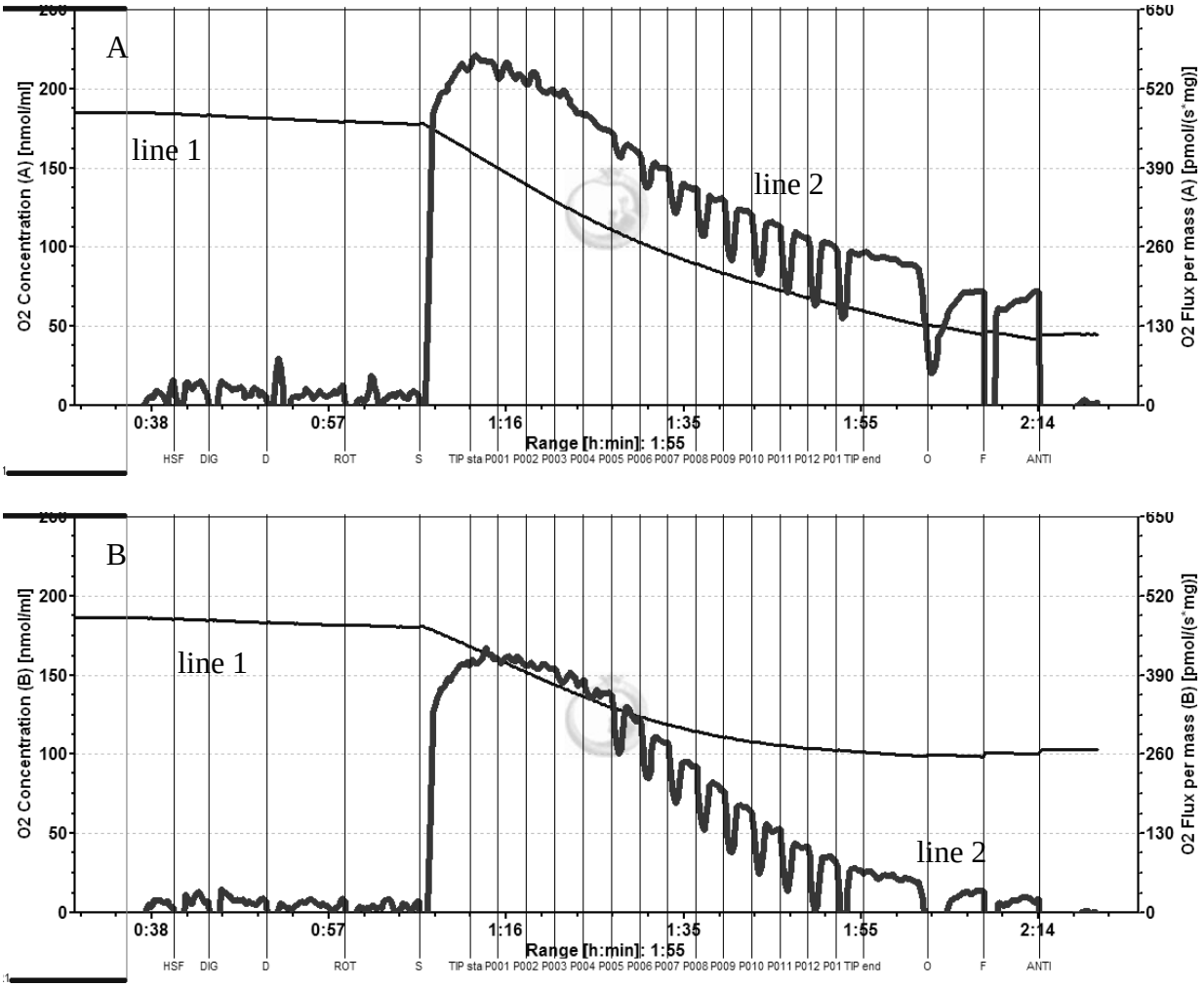


Fig. 4 Typical graphs from oxygraph presenting drug-induced inhibition of oxygen consumption of brain mitochondria. Medium-titrated (A) and chlorpromazine-titrated (B) samples with respiration controlled through complex II. Vertical lines label addition of components: HSF - crude mitochondrial fraction; DIG - digitonin; D - ADP; ROT - rotenone, S - succinate; P001-P013 - single titrations; O - oligomycin; F – FCCP (*p*-trifluoromethoxyphenylhydrazone); ANTI - antimycin A. Line 1 demonstrates concentration of oxygen in a sample. Line 2 represents kinetic of oxygen consumption (negative derivation of the curve 1).

3.5 Respiratory rate in platelets of depressive persons

Subjects

Patients with an ICD-10 diagnosis of depression were recruited from Department of Psychiatry of First Faculty of Medicine and General University Hospital in Prague. The patients were asked to complete questionnaires concerning medical history, personal habits, and use of medications. Severity of depression was evaluated by a Hamilton Rating Scale for Depression (HRSD-21), Clinical Global Impression - Severity scale (CGI-S) and Clinical Global Impression - Improvement scale (CGI-I). Depressive patients were tested both before treatment and after several weeks of treatment with antidepressants. Positive depressive symptomatology was recognized at HRSD-21 score reached value > 10, negative depressive symptomatology at HRSD-21 $\leq$ 10. Control group consisted of normal healthy volunteers. Patients before the blood taking were on empty stomach, without the use of cigarettes and coffee, and before the administration of medicaments. The study was approved by the Ethics Committee of General University Hospital, Prague. All patients and controls gave written, informed consent to participate in the study.

Human platelets

Venous blood withdrawal was performed using Vacutainer® (Becton, Dickinson and Company) blood collection tubes with sodium citrate or EDTA as anticoagulant. To isolate platelets, blood samples were immediately centrifuged at 200 g for 15 min at 25 °C, thus obtaining platelet rich plasma (PRP) in supernatant. Platelets in PRP were counted using Bürker chamber. PRP with sodium citrate were diluted by Krebs-Henseleit medium without Ca^{2+} (KH medium) consisted of 118 mmol/L NaCl, 4.7 mmol/L KCl, 1.2 mmol/L KH_2PO_4, 1.2 mmol/L $MgSO_4.7H_2O$, 25 mmol/L $NaHCO_3$, and 11.1 mmol/L glucose, pH 7.4) to concentration 200.10^6/mL and used for measurement of respiration of intact platelets. PRP with EDTA was centrifuged at 1500 g for 10 min at 25 °C; the pellet was resuspended in the same volume of MiR05 medium and used for measurement of respiration of permeabilized platelets.

High-resolution respirometry

Activity of mitochondrial respiratory system was measured at 37 °C in high-resolution oxygraph as described above. Since the rate of respiration in the cell is not maximal, the

respiration rate was measured in presence of an optimal concentration of uncoupler, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP), which induces the noncoupled state of maximal respiratory rate and enable measurement of the capacity of the electron transfer system (ETS). Intrinsic uncoupling and dyscoupling may be evaluated by the flux control ratio (FCR) between nonphosphorylating respiration (LEAK), caused by electron flow coupled to proton pumping to compensate for proton leaks, and ETS capacity (Pesta and Gnaiger, 2012).

Data were collected and analyzed using software DatLab 4.3 (Oroboros Instruments, Innsbruck, Austria). Oxygen solubility factor relative to distilled water for buffers was set to 0.92 (Sjövall et al., 2010). Respiratory rates (oxygen fluxes) were expressed either as pmol O_2 consumed per second relative to one milligram of protein in the sample of crude brain mitochondria (pmol/mg.s) or as pmol O_2 consumed per second relative to one million of platelets in the sample of intact or permeabilized cells (pmol/s.10^6 platelets).

Respiration of intact platelets

Mitochondrial respiration was measured in intact platelets (Sjövall et al., 2010). From platelet suspension in plasma with KH medium, 2.0 ml aliquot, containing 400.10^6 platelets, was incubated for 30 min at 37 °C with continuous stirring in measuring chamber of high-resolution respirometer with unrestricted access of the air. Following stabilizing of oxygen concentration in suspension the chamber was closed by stopper and mitochondria were allowed to stir for approximately 5 min until signal from oxygraph was stabilized; physiological coupling state controlled by platelet energy demands on OXPHOS was measured as respiration of intact platelets without any additions (***ROUTINE'***). Respiration independent of ADP phosphorylation was measured after addition 1 µL (2 µg/mL) oligomycin, the ATP synthase inhibitor (oligomycin induced state 4, ***LEAK'***). Maximal capacity of the electron transfer system (***ETS'***) in the noncoupled state was achieved by titration with the protonophore FCCP (10-80 µmol/L). Finally, mitochondrial respiration was inhibited by 5 µL (2.5 µmol/L) rotenone (complex I inhibitor) and 10 µL (2.5 µg/mL) antimycin A (complex III inhibitor) and residual oxygen consumption (***ROX***) was measured to be subtracted from all other respiratory states.

Following ***ROX*** subtraction from ***ROUTINE'***, ***LEAK'*** and ***ETS'***, flux control ratios were calculated as ***ROUTINE/ETS***, ***LEAK/ETS***, (***ROUTINE-LEAK)/ETS*** and ***ROX/ETS'*** (Gnaiger, 2009; Sjövall et al., 2010; Pesta and Gnaiger, 2012) to determine relative contribution of the

different respiratory states (i.e. respiratory control independent of mitochondrial content and cell size).

Data record of measurement in intact platelets is exemplified in Fig. 5.

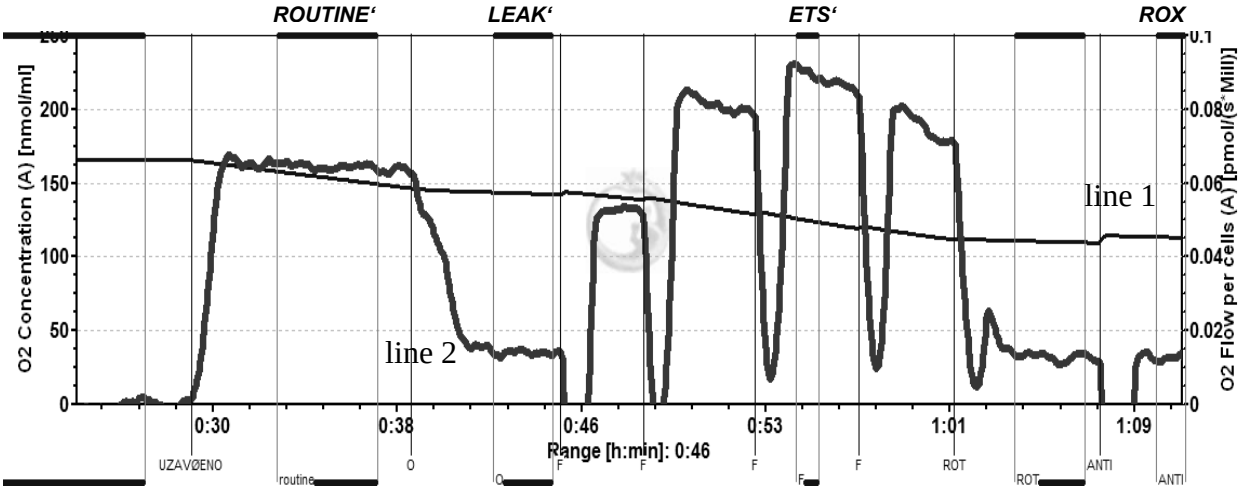


Fig. 5 Typical curve from oxygraph presenting oxygen consumption in intact platelets. Line 1 demonstrates oxygen concentration in a sample; line 2 demonstrates oxygen consumption (negative derivation of oxygen concentration). Vertical lines indicate additions of single components: O - oligomycin (induction of LEAK state); F – FCCP (*p*-trifluoromethoxyphenylhydrazone; maximum of electron transport system capacity); ROT - rotenone; ANTI - antimycin A.

Respiration of permeabilized platelets

Respiration was measured using mitochondrial substrates in platelets that have been permeabilized with digitonin. This permeabilization allows measuring the respiratory rate and respiratory control ratios with different substrates thereby obtaining information on coupling sites of OXPHOS. NADH-related substrates (malate, pyruvate, glutamate) plus succinate supply enable reconstitute the citric acid cycle function in permeabilized platelets or isolated mitochondria, with convergent complex I and complex II input (Gnaiger, 2009).

Experimental protocol was adapted from Sjövall et al. (2010). From platelet suspension in MiR05, 2.0 ml aliquot, containing at the average 900.10^6 platelets, was incubated for 30 min at 37 °C with continuous stirring in measuring chamber of high-resolution respirometer with unrestricted access of the air. Following stabilizing of oxygen concentration in suspension the chamber was closed by stopper and mitochondria were allowed to stir for approximately 5 min until signal from oxygraph was stabilized and respiration of intact platelets without any additions

was determined (***ROUTINE'***). Platelets were permeabilized with 10 µL digitonin (50 µg/mL). Respiration through complex I was initiated by adding 5 µL malate (5 mmol/L) and 5 µL pyruvate (5 mmol/L); then state 3_{CI} respiration was induced by the addition of 4 µL ADP (1.0 mmol/L) and 5 µL glutamate (5 mmol/L) (***OXPHOS$_I$'***). After signal stabilizing 20 µL succinate (10 mmol/L) was added to activate respiratory through complex II and maximal OXPHOS capacity by convergent input through both complex I and complex II (state 3_{CI+II}) was measured (***OXPHOS$_{I+II}$'***). Respiration independent of ADP phosphorylation was measured after addition 1 µL (2 µg/mL) oligomycin (oligomycin induced state 4, ***LEAK'***) and maximal capacity of the electron transfer system (***ETS'***) was obtained by titration with the protonophore FCCP (1-3 µmol/L). Mitochondrial respiration through complex I was inhibited by 1 µL rotenone (0.5 µmol/L) and the ETS capacity related to complex II was determined (***ROT'***). Finally, 5 µL antimycin A (1.25 µg/mL) was added and residual oxygen consumption (***ROX***) was measured to be subtracted from all other respiratory states.

Following ***ROX*** subtraction, respiratory flux control ratios were calculated as ***ROUTINE/ETS***, ***OXPHOS$_I$/ETS***, ***OXPHOS$_{I+II}$/ETS***, ***LEAK/ETS***, ***ROT/ETS***, ***LEAK/ OXPHOS$_I$***, ***LEAK/ OXPHOS$_{I+II}$*** (inverse of the conventional respiratory control ratio), and ***ROX/ETS'*** (Gnaiger, 2009; Sjövall et al., 2010; Pesta and Gnaiger, 2012) to determine relative contribution of the different respiratory states.

Data record of measurement in permeabilized platelets is exemplified in Fig. 6.

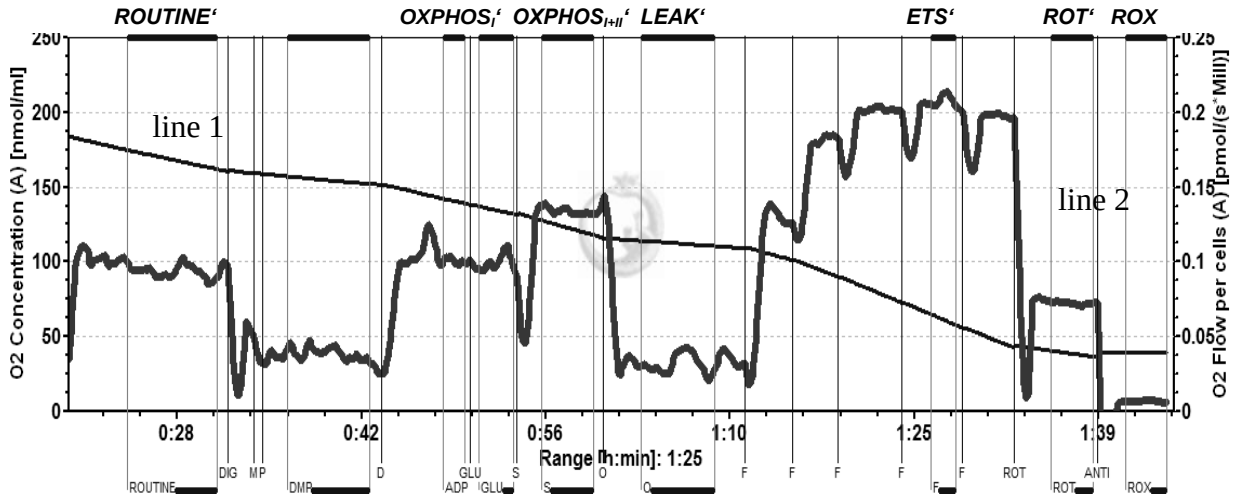


Fig. 6 Typical curves from oxygraph presenting oxygen consumption in permeabilized platelets. Line 1 represents oxygen concentration in a sample; line 2 represents oxygen consumption (negative derivation of curve 1). Vertical lines indicate additions of single components: DIG - digitonin (for membrane permeabilization), M - malate; P - pyruvate; D - ADP; GLU - glutamate; S - succinate; O - oligomycin (induction of LEAK state); F - FCCP (*p*-trifluoromethoxyphenylhydrazone; maximum of electron transport system capacity); ROT - rotenone; ANTI - antimycin A.

Data analysis

Data were collected using the software displaying the oxygen flux and real-time oxygen consumption (DatLab software 4.3b, Oroboros Instruments, Austria). All data presented are expressed as the mean ± standard deviation. Results were analysed by STATISTICA (data analysis software system, version 10.0, StatSoft, Inc., Tulsa, OK, USA). Mann-Whitney U test (a nonparametric test for evaluation of independent samples from the same distribution) was used to calculate test statistics in order to compare values obtained from patients in depressive episode, patients after a long-term treatment and controls.

4. Results

4.1 Drug effects on enzymes of citric acid cycle and respiratory chain complexes

Activities of respiratory chain complexes were mostly decreased owing to tested antidepressants and mood stabilizers; the most affected were complex I and complex IV (Fig. 7). Statistically significant decrease of complex I activity was found for desipramine, amitriptyline, imipramine, citalopram, mirtazapine and valproate. Activity of complex II was significantly decreased by amitriptyline and imipramine. Activity of complex IV was significantly decreased for desipramine, amitriptyline, imipramine, mirtazapine, tianeptine, lithium, valproate and olanzapine.

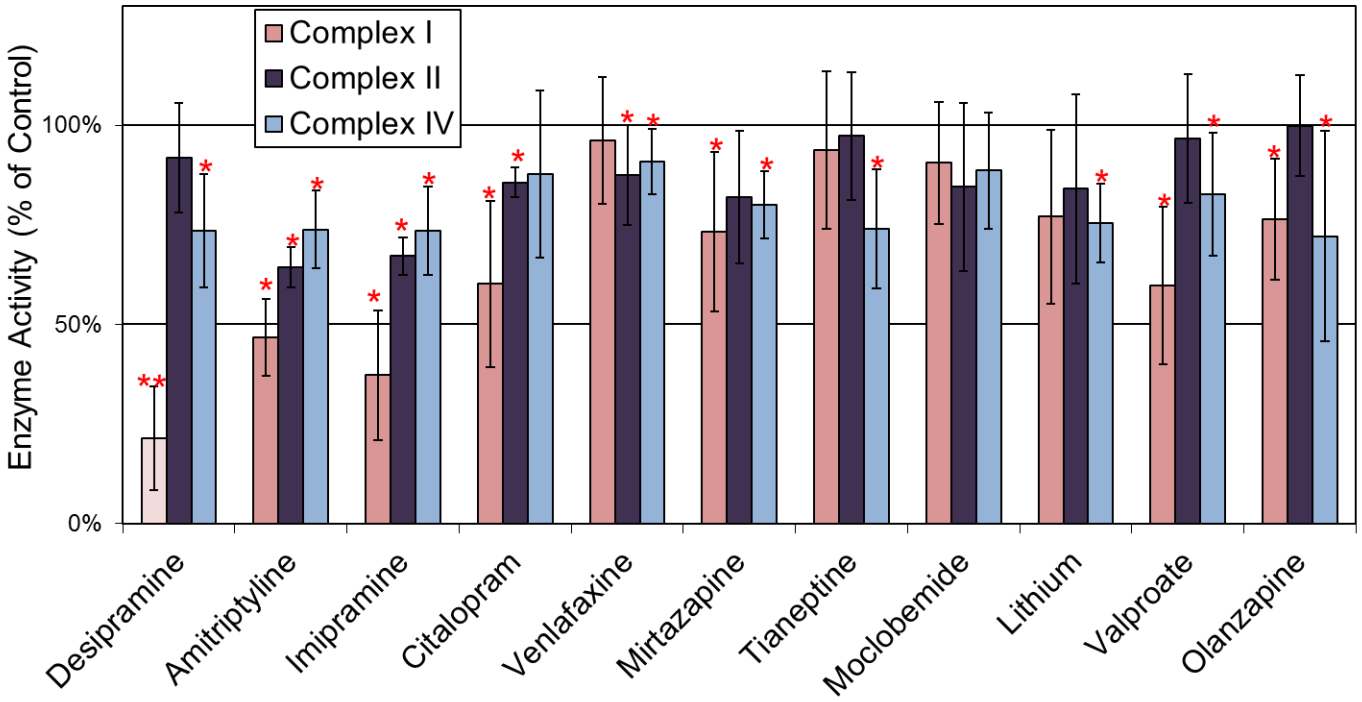


Fig. 7 Effects of antidepressants and mood stabilizers on activities of the respiratory chain complexes I, II, IV in a brain crude mitochondrial fraction. The samples were incubated with drugs at 30 °C for 30 minutes and enzyme kinetics were measured spectrophotometrically as described in the section "Material and Methods". Relative activities are displayed (100 % = control sample without the drug). Values are means ± standard deviation of at least five independent measurements. Comparisons between controls and samples with drug were performed using the Wilcoxon matched pairs test (*$p<0.05$, **$p<0.01$).

Considering very high final drug concentrations in samples the values showed on Fig. 7 conform to residual activities of enzyme complexes. The inhibitory curve for inhibition of the complex I by desipramine illustrates the relevance of residual activity (Fig. 8).

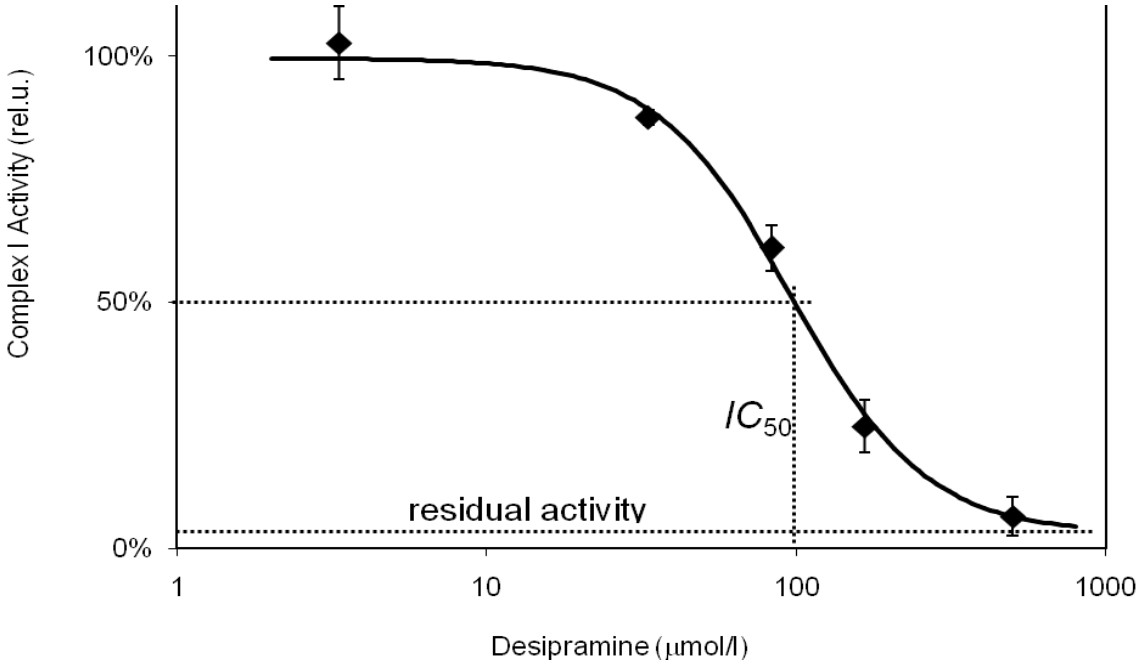


Fig. 8 Inhibition of basal NADH dehydrogenase (complex I) activity by desipramine in a brain crude mitochondrial fraction. Concentration-response curve is displayed as plot of the initial activity of complex I against the desipramine concentration. The samples were incubated with drugs at 30 °C for 30 minutes as described in "Material and Methods". The reaction was started by the addition of 33 μmol/l coenzyme Q_1 and measured for 10 min; afterwards rotenone was added (in final concentration 50 μmol/l) and the inhibited rate was measured for further 2 min. The control samples were measured simultaneously. Values are mean ± standard deviation of three independent measurements. Line represent the best fitted curve using the four-parameter logistic function (median effective concentration IC_{50} = 96.3 ± 9.7 μmol/l, residual activity = 3.05 ± 0.55 %, and Hill slope = 2.00 ± 0.35).

Potencies of tested drug in affecting of CS and SDH activity are summarized in the Table 4. Except for mirtazapine and moclobemide all tested drugs slightly increased CS activity; however, the increase was statistically significant only for citalopram, tianeptine and olanzapine.

Table 4 Effects of antidepressants and mood stabilizers on citrate synthase (CS) and succinate dehydrogenase (SDH) activity in a brain crude mitochondrial fraction.

Drug	*Activity CS (% of Control)*	*N(CS)*	*Activity SDH (% of Control)*	*N(SDH)*
Desipramine	105 ± 13	9	96 ± 17	4
Amitriptyline	109 ± 13	8	*85 ± 17	6
Imipramine	106 ± 11	5	105 ± 19	4
Citalopram	*116 ± 12	6	82 ± 16	3
Venlafaxine	115 ± 22	6	111 ± 41	5
Mirtazapine	96 ± 24	6	95 ± 9	6
Tianeptine	*120 ± 21	9	106 ± 14	3
Moclobemide	98 ± 4	5	104 ± 12	3
Lithium	108 ± 12	8	84 ± 23	5
Valproate	109 ± 10	6	-	-
Olanzapine	*129 ± 16	8	-	-

The samples were incubated with drugs at 30 °C for 30 minutes. After the incubation, CS activity was initiated by the addition of 0.5 mmol/l oxaloacetate and was measured for 3 minutes at 412 nm. After the incubation, reaction for the determination of SDH activity was initiated by addition of 0.3% iodotetrazonium chloride - final concentration 2 mmol/l) and was continuously monitored for 10 minutes at 500 nm. The control samples (without the drug) were measured simultaneously. The effect of antidepressants and mood stabilizers on the enzyme activity was expressed as percentage of activity of the control sample.

Values are mean ± standard deviation; *N* = number of independent measurements; *$p<0.05$, i.e. the Wilcoxon matched pairs test was significant at the 0.05 level.

4.2 Drug effects on mitochondrial respiratory rate

The effects of antidepressants (amitriptyline, fluoxetine, tianeptine), mood stabilizers (lithium carbonate, sodium valproate, and olanzapine), ketamine, and chlorpromazine on respiration rate in pig brain mitochondria were assessed and compared with effect of chlorpromazine. Potency of tested drugs in inhibiting mitochondrial respiratory rate is summarized in the Tables 5 and 6. Among the tested antidepressants, fluoxetine and tianeptine were the most potent inhibitors of respiration supported by substrates for electron supply through complex I (Fig. 9, Table 5). Tianeptin, but not fluoxetine was potent inhibitor of respiration supported by substrates for

electron supply through complex II (Fig. 10, Table 6). Ketamin and mood stabilizers did not affect markedly mitochondrial respiration regardless of substrate used. Hill slope of all inhibitory curves was significantly higher than 1; it indicates positively cooperative reaction. High residual activity at high drug concentration was observed for all drugs (Tables 5 and 6, Figs. 9 and 10); it indicates that only partial inhibition of respiratory rate occurs.

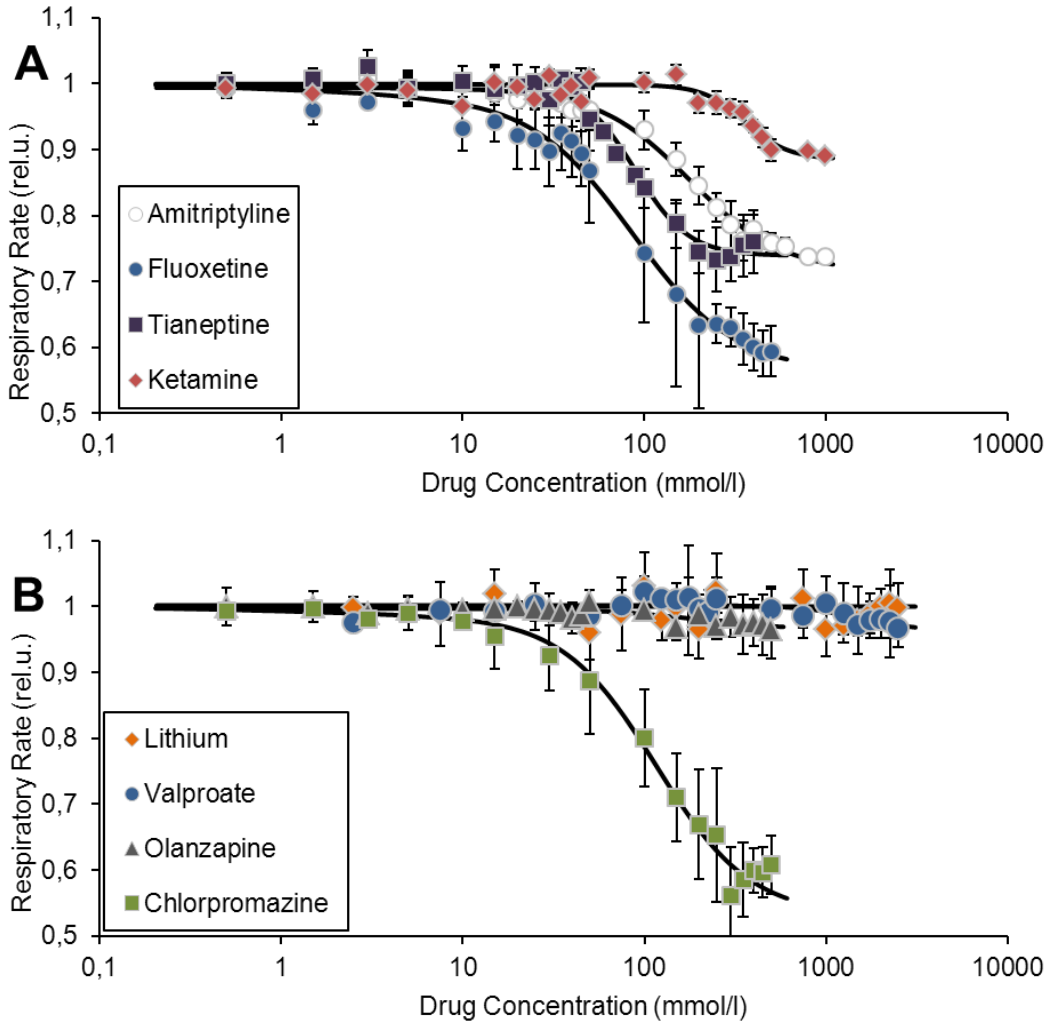


Fig. 9. Drug-induced inhibition of respiratory rate in pig brain mitochondria incubated with substrates for electron supply through complex I. Changes in respiratory rate induced by antidepressant (A) and mood stabilizers (B) were measured. Dose-response curves are displayed as plots of the respiratory rate against the drug concentration. The samples were continuously stirred and incubated at 37°C; titration with drugs was achieved in 3 min intervals. Following subtraction of residual oxygen consumption from respiratory rates at different drug/medium concentrations, differences among drug-titrated and medium-titrated samples were calculated and relative drug-induced changes in respiratory rate were determined, supposing that relative respiratory rate equals to 1 at zero drug concentration. Median effective concentrations (IC_{50}) and Hill slope were calculated using nonlinear regression analysis software (Table 1). Values are means from at least 4 independent measurements ± standard deviation. Lines represent the best fitted curves using the four-parameter logistic function.

Table 5 Drug-induced inhibition of respiratory rate in pig brain mitochondria incubated with substrates for electron supply through complex I

Drug	*IC_{50} (µmol/l)*	*Hill slope*	*N*	*Residual (rel.u.)*
Amitriptyline	178.2 ± 9.4	1.70 ± 0.12	10	0.714
Fluoxetine	86.2 ± 9.5	1.53 ± 0.19	8	0.562
Tianeptine	88.9 ± 2.6	2.95 ± 0.23	9	0.739
Ketamine	361.6 ± 21.5	3.70 ± 0.70	5	0.886
Lithium	Nd	Nd	8	1.000
Valproate	Nd	Nd	7	0.964
Olanzapine	Nd	Nd	9	0.964
Chlorpromazine	115.9 ± 10.7	1.67 ± 0.19	8	0.530

Values are means ± standard deviation; *IC_{50}* - half maximal inhibitory concentration; *Hill slope* - characterizes the slope of the curve at its midpoint and it is used in determining the degree of cooperativity of the ligand binding to the enzyme; *N* - number of measurement; *Residual* - residual activity at high drug concentration.

Table 6 Drug-induced inhibition of respiratory rate in pig brain mitochondria incubated with substrates for electron supply through complex II

Drug	*IC_{50} (µmol/l)*	*Hill slope*	*N*	*Residual (rel.u.)*
Amitriptyline	462 ± 25	2.58 ± 0.22	10	0.662
Fluoxetine	266.2 ± 8.9	3.37 ± 0.30	9	0.540
Tianeptine	67.4 ± 4.9	2.39 ± 0.36	8	0.717
Ketamine	Nd	Nd	4	1.000
Lithium	Nd	Nd	8	1.000
Valproate	Nd	Nd	7	0.956
Olanzapine	419 ± 213	2.16 ± 0.77	7	0.878
Chlorpromazine	263 ± 33	2.38 ± 0.45	10	0.395

Values are means ± standard deviation; *IC_{50}* - half maximal inhibitory concentration; *Hill slope* - characterizes the slope of the curve at its midpoint and it is used in determining the degree of cooperativity of the ligand binding to the enzyme; *N* - number of measurement; *Residual* - residual activity at high drug concentration.

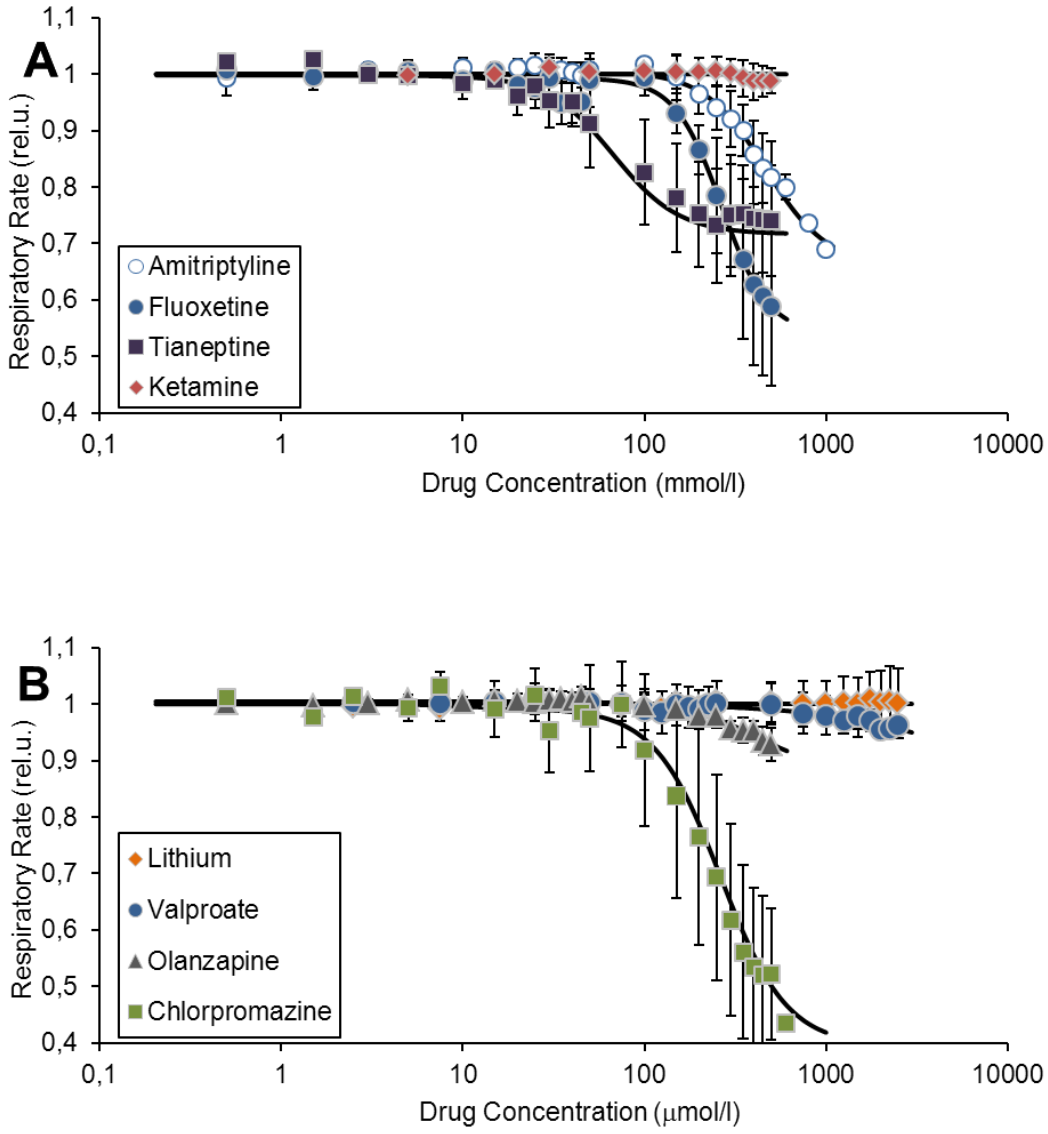


Fig. 10. Drug-induced inhibition of respiratory rate in pig brain mitochondria incubated with substrates for electron supply through complex II. Changes in respiratory rate induced by antidepressant (A) and mood stabilizers (B) were measured. Dose-response curves are displayed as plots of the respiratory rate against the drug concentration. The samples were continuously stirred and incubated at 37 °C; titration with drugs was achieved in 3 min intervals. Following subtraction of residual oxygen consumption from respiratory rates at different drug/medium concentrations, differences among drug-titrated and medium-titrated samples were calculated and relative drug-induced changes in respiratory rate were determined, supposing that relative respiratory rate equals to 1 at zero drug concentration. Median effective concentrations (IC_{50}) and Hill slope were calculated using nonlinear regression analysis software (Table 6). Values are means from at least 4 independent measurements ± standard deviation. Lines represent the best fitted curves using the four-parameter logistic function.

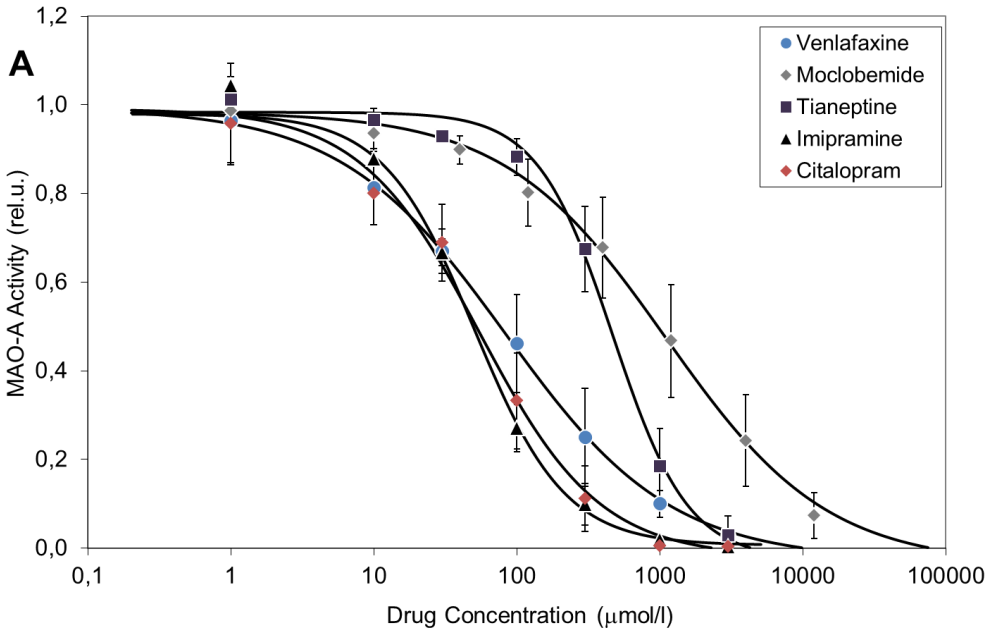
4.3 Drug effects on monoamine oxidase activity

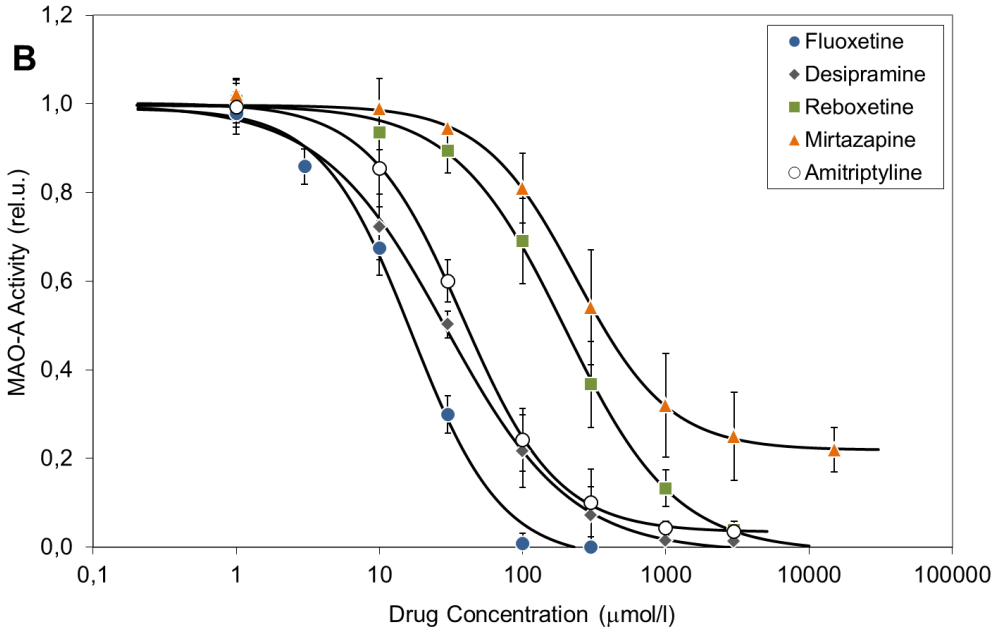
The effects of antidepressants (desipramine, amitriptyline, imipramine, fluoxetine, citalopram, venlafaxine, reboxetine, mirtazapine, tianeptine, moclobemide) an mood stabilizers (lithium, valproate, olanzapine) on MAOs activity and nature of the interaction in pig brain mitochondria were assessed in comparison with iproniazid, pargyline, clorgyline. As was to be expected, the mitochondria treatment with iproniazid, pargyline or clorgyline resulted in decrease of the MAOs activity at nanomolar to micromolar drug concentrations (Figs. 11C, 12C). IC_{50} values were determined by analysing dose-response curves of the MAO activity against the drug concentration. It must be noted that irreversible inhibitors (iproniazid, pargyline, clorgyline) displays time-dependent inhibition and its potency cannot be correctly characterized by IC_{50} value.

Potency of drug tested in inhibiting MAO-A activity is summarized in the Table 7. Most of drugs were relatively potent in inhibiting MAO-A activity, whereas MAO-A was not fully inhibited even at high mirtazapine or olanzapine concentrations (lower efficacy). The residual activities of MAO-A were about 21.9 %, and 29.1 % with mirtazapine and olanzapine, respectively (Fig. 11). Amitriptyline, imipramine, fluoxetine, mirtazapine and tianeptine displayed positive cooperativity of the binding to MAO-A. Venlafaxine, moclobemide and olanzapine displayed negative cooperativity. A Hill coefficient of 1 indicates completely independent binding for desipramine and citalopram.

The effects of the same drugs also were evaluated on pig brain mitochondrial MAO-B activity using PEA as substrate (Table 8). Most of drugs were relatively potent in inhibiting MAO-B activity. Similarly to MAO-A, MAO-B was not fully inhibited even at high mirtazapine or olanzapine concentrations; however, lower efficacy of inhibition was found for venlafaxine and amitriptyline. The residual activities of MAO-B were about 29.0 %, 32.2 %, 58.5 %, 17.3 % and 39.9 % with venlafaxine, amitriptyline, olanzapine, and mirtazapine, respectively (Fig. 12). Fluoxetine, venlafaxine, amitriptyline and tianeptine displayed positive cooperativity of the binding to MAO-B; olanzapine displayed negative cooperativity.

Lithium and valproate are very weak inhibitors of both isoforms of MAO. Moclobemide does not inhibit MAO-B and it is a weak *in vitro* inhibitor of MAO-A.


A
Venlafaxine
Moclobemide
Tianeptine
Imipramine
Citalopram
MAO-A Activity (rel.u.)
1,2
1,0
0,8
0,6
0,4
0,2
0,0
0,1
1
10
100
1000
10000
100000
Drug Concentration (µmol/l)


B
Fluoxetine
Desipramine
Reboxetine
Mirtazapine
Amitriptyline
MAO-A Activity (rel.u.)
1,2
1,0
0,8
0,6
0,4
0,2
0,0
0,1
1
10
100
1000
10000
100000
Drug Concentration (µmol/l)

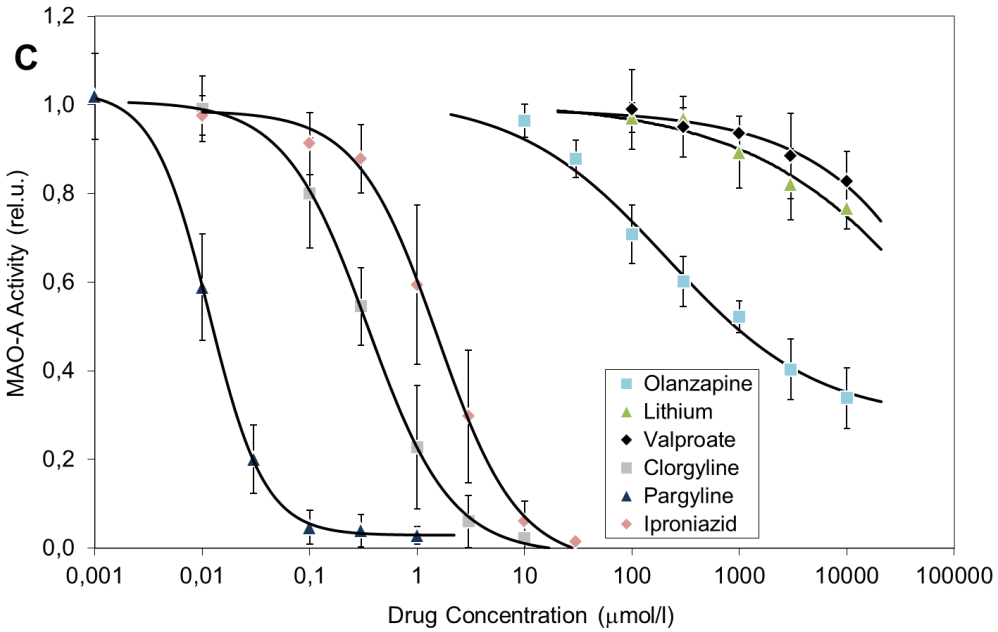


Fig. 11 Inhibition of basal MAO-A activity by antidepressants in crude mitochondrial fraction isolated from cortex of pig brain. Dose-response curves are displayed as plots of the initial activity of MAO-A against the antidepressant concentration. The samples were incubated with drugs at 37 °C for 60 minutes and the reaction was started by the addition of 3.2 µmol/l [^{14}C]serotonin. Following incubation at 37 °C for 30 minutes, the reaction was stopped by the addition of hydrochloric acid. The samples were measured in doublets and blank values were deducted. Median effective concentrations (IC_{50}) were calculated using nonlinear regression analysis software (Table 7). Values are means ± standard deviation. Lines represent the best fitted curves using the four-parameter logistic function.

To determine the mechanism of MAO inhibition by the antidepressants and mood stabilizers, the effects of various concentrations of these drugs on MAO kinetic were studied. Enzyme kinetic equations for competitive, noncompetitive, uncompetitive and mixed type of inhibition were tested and the best model was chosen.

When the kinetics of the interactions of drugs with the enzyme were assessed, SigmaPlot calculation of MAO-A kinetics confirmed that the interaction was competitive for moclobemide and olanzapine only (i.e. apparent increases in K_m values with no changes in V_{max} values in the presence of the inhibitor), noncompetitive for fluoxetine, imipramine, citalopram, venlafaxine and mirtazapine (i.e. apparent decreases in V_{max} values with no changes in K_m values in the presence of the inhibitor), mixed for desipramine and amitriptyline (i.e. apparent decreases in V_{max} values with the change in K_m values in the presence of the inhibitor), and uncompetitive for tianeptine (i.e. apparent decreases in V_{max} values with decrease in K_m values in the presence of the inhibitor) (Table 7).

Table 7 Inhibition of MAO-A activity in brain crude mitochondrial fraction

Drug	*IC_{50} µmol/l*	*Hill slope*	*Mechanism of inhibition*	*N*
Fluoxetine	16.8 ± 1.0	1.38 ± 0.10	Noncompetitive	5
Desipramine	29.2 ± 2.8	1.00 ± 0.09	Mixed	7
Amitriptyline	38.2 ± 2.9	1.31 ± 0.11	Mixed	8
Imipramine	50.5 ± 3.5	1.37 ± 0.10	Noncompetitive	6
Citalopram	55.0 ± 5.4	1.03 ± 0.09	Noncompetitive	8
Venlafaxine	83.5 ± 12.6	0.75 ± 0.08	Noncompetitive	8
Olanzapine	212 ± 71	0.62 ± 0.10	Competitive	5
Mirtazapine	235 ± 30	1.33 ± 0.21	Noncompetitive	9
Tianeptine	469 ± 39	1.65 ± 0.17	Uncompetitive	7
Moclobemide	1105 ± 101	0.77 ± 0.05	Competitive	10
Lithium	>10000			6
Valproate	>10000			6
Pargyline	0.01152 ± 0.00067	1.68 ± 0.18	Irreversible	12
Clorgyline	0.347 ± 0.041	1.12 ± 0.13	Irreversible	14
Iproniazid	1.55 ± 0.12	1.16 ± 0.10	Irreversible	16

The samples were incubated with drugs at 37 °C for 60 minutes and the reaction was started by the addition of 3.2 µmol/l [^{14}C]serotonin; following incubation at 37 °C for 30 minutes, the reaction was stopped by the addition of hydrochloric acid. The samples were measured in doublets and blank values were deducted.

Values are means ± standard deviation. IC_{50} = drug concentration that is required for 50% inhibition of enzyme activity; Hill slope = describes the cooperativity of the drug binding; *N* = number of measurements.

The effects of the same drugs also were evaluated on kinetic parameters of pig brain mitochondrial MAO-B activity. SigmaPlot calculation of MAO-B kinetics confirmed that the interaction was noncompetitive for fluoxetine, olanzapine, citalopram and desipramine, mixed for amitriptyline, imipramine and mirtazapine, and uncompetitive for venlafaxine and tianeptine (Table 8).

Both lithium and valproate are very weak MAOIs; mechanism of MAO-A or MAO-B inhibition at high drug concentrations appears to be noncompetitive.

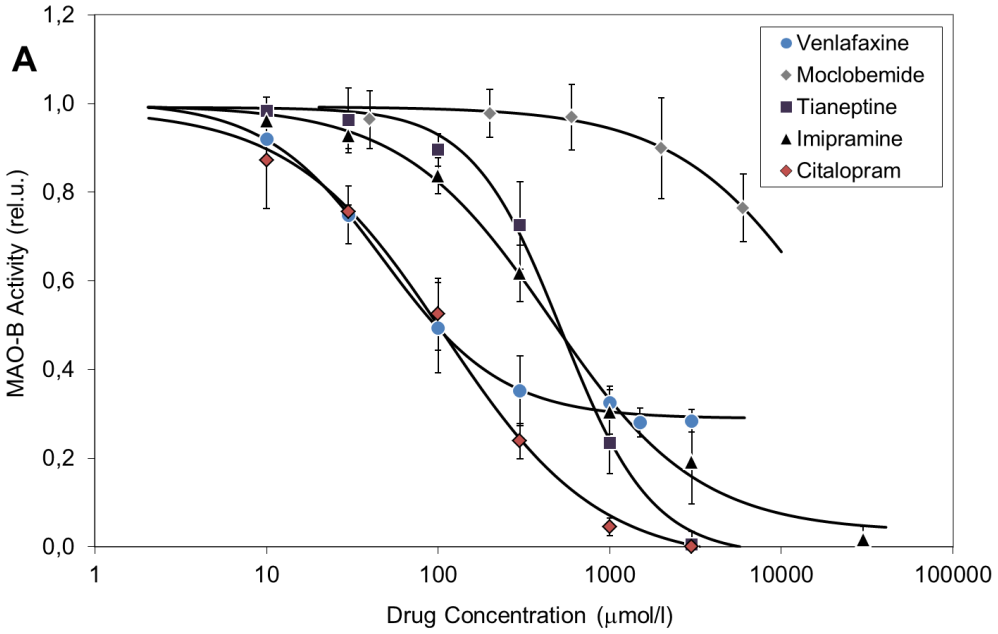
Competitive inhibition of MAOs by drugs means that their inhibition constants K_i must be calculated using Cheng-Prusoff equation; in all other cases K_i approximately equals to IC_{50} in the Tables 7 and 8.

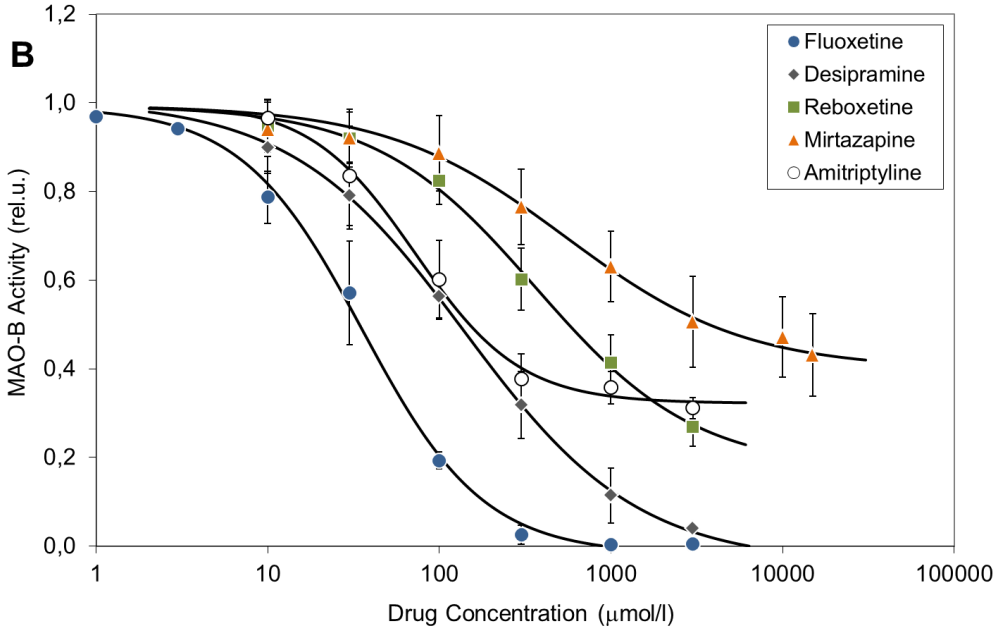
Table 8 Inhibition of MAO-B activity in brain crude mitochondrial fraction

Drug	*IC_{50} µmol/l*	*Hill slope*	*Mechanism of inhibition*	*N*
Fluoxetine	35.7 ± 3.1	1.23 ± 0.12	Noncompetitive	4
Venlafaxine	48.0 ± 4.8	1.26 ± 0.13	Uncompetitive	10
Amitriptyline	72.1 ± 8.0	1.41 ± 0.19	Mixed	8
Olanzapine	79 ± 44	0.66 ± 0.21	Noncompetitive	9
Citalopram	110 ± 11	0.975 ± 0.089	Noncompetitive	5
Desipramine	140 ± 18	0.859 ± 0.085	Noncompetitive	7
Imipramine	452 ± 51	1.006 ± 0.098	Mixed	5
Tianeptine	526 ± 34	1.65 ± 0.14	Uncompetitive	7
Mirtazapine	546 ± 108	0.83 ± 0.12	Mixed	15
Moclobemide	>10000			7
Lithium	>10000			6
Valproate	>10000			6
Pargyline	0.00820 ± 0.00033	1.311 ± 0.057	Irreversible	11
Clorgyline	0.346 ± 0.019	1.118 ± 0.065	Irreversible	6
Iproniazid	1.210 ± 0.067	1.156 ± 0.064	Irreversible	10

The samples were incubated with drugs at 37 °C for 60 minutes and the reaction was started by the addition of 10 µmol/l [^{14}C]phenylethylamine; following incubation at 37 °C for 1 minute, the reaction was stopped by the addition of hydrochloric acid. The samples were measured in doublets and blank values were deducted.

Values are means ± standard deviation. IC_{50} = drug concentration that is required for 50% inhibition of enzyme activity; Hill slope = describes the cooperativity of the drug binding; *N* = number of measurements.


A
Venlafaxine
Moclobemide
Tianeptine
Imipramine
Citalopram
MAO-B Activity (rel.u.)
1,2
1,0
0,8
0,6
0,4
0,2
0,0
1
10
100
1000
10000
100000
Drug Concentration (µmol/l)


B
Fluoxetine
Desipramine
Reboxetine
Mirtazapine
Amitriptyline
MAO-B Activity (rel.u.)
1,2
1,0
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Drug Concentration (µmol/l)

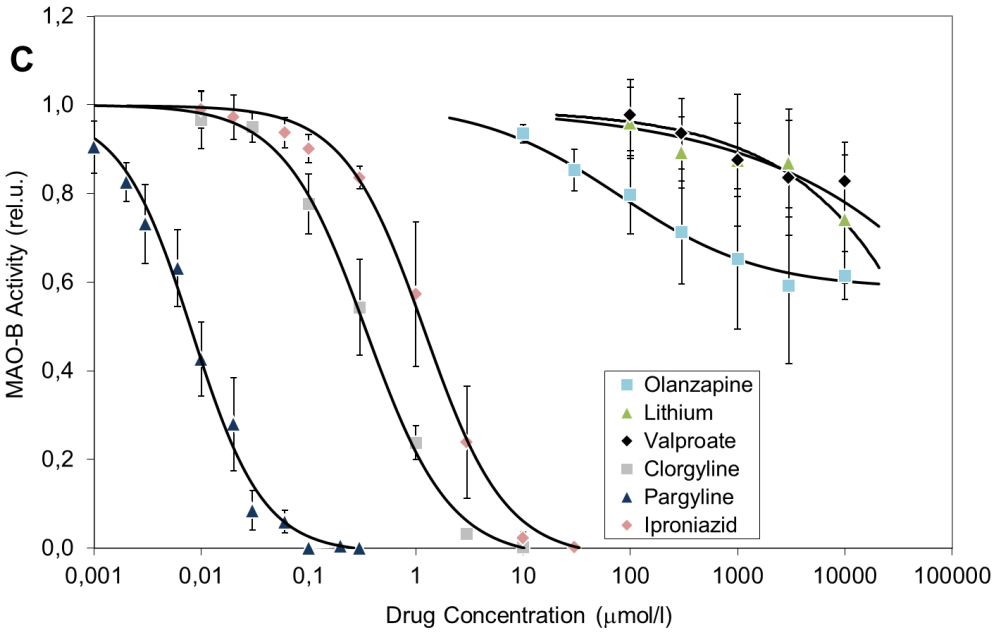

Fig. 12 Inhibition of basal MAO-B activity by antidepressants in crude mitochondrial fraction isolated from cortex of pig brain. Dose-response curves are displayed as plots of the initial activity of MAO-B against the antidepressant concentration. The samples were incubated with drugs at 37 °C for 60 minutes and the reaction was started by the addition of 10 μmol/l [^{14}C]phenylethylamine. Following incubation at 37 °C for 1 minute, the reaction was stopped by the addition of hydrochloric acid. The samples were measured in doublets and blank values were deducted. Median effective concentrations (IC_{50}) were calculated using nonlinear regression analysis software (Table 8). Values are means ± standard deviation. Lines represent the best fitted curves using the four-parameter logistic function.

4.4 Respiratory rate in platelets of depressive persons

11 depressive patients before and after long-term treatment and 16 controls were compared in the preliminary study of association of depressive disorder with changes in mitochondrial respiration in intact or in permeabilized platelets.

In intact platelets, physiological respiration (0.061 ± 0.010) and maximal FCCP-titrated capacity of ETS (0.080 ± 0.016) were unchanged before the treatment, but were decreased after the treatment, compared to controls (0.074 ± 0.019 and 0.105 ± 0.026, respectively). Similarly, state 4 (LEAK) was significantly decreased after the treatment (0.0015 ± 0.0029), compared both to controls (0.0043 ± 0.0048) and to state before the treatment (0.0060 ± 0.0038). Capacity of ETS after the inhibition of complex I (CII_{ETS}) by rotenone was increased in patients before the

treatment (0.0076 ± 0.0054) compared to both controls (0.0042 ± 0.0039) and patients after treatment (0.0033 ± 0.0023) (Figure 13A).

Net physiological respiration/ETS ratio was significantly increased in depressive patients both before treatment (0.757 ± 0.161) and after the treatment (0.755 ± 0.135) compared to healthy controls (0.656 ± 0.97). Residual oxygen consumption/ETS ratio was significantly higher in depressive patients after treatment (0.202 ± 0.077) compared to controls (0.139 ± 0.040). Oppositely, LEAK/ETS ratio was significantly increased before the treatment (0.069 ± 0.044) compared to state after the treatment (0.016 ± 0.033). Demonstrated in Figure 13B.

Measurements **in permeabilized platelets** have shown similar but nonsignificant results. Routine (initial) respiration was decreased before the treatment as well as after the treatment compared to controls. Respiration stimulated by complex I substrates (ADP stimulated respiration) was increased in depressive patients before the treatment (0.146 ± 0.038) compared to controls (0.119 ± 0.041) and state after treatment (0.111 ± 0.046). Nonsigificant decrease of respiratory rate in permeabilized platelets of depressed subjects after treatment was observed also when respiration was stimulated by complex II (succinate as substrate) or when capacity of ETS was measured. Respiration with complex I inhibition did not reveal any changes. Depicted in Figure 14A.

FCR related to initial respiration was significantly decreased in depressive patients before treatment (0.423 ± 0.083) compared to controls (0.557 ± 0.134). FCR related to ADP (complex I) stimulated respiration was increased before treatment (0.577 ± 0.099) compared to controls (0.489 ± 0.080). LEAK/ETS ratio was decreased before treatment (0.140 ± 0.031) compared to controls (0.181 ± 0.051) (Figure 14B).

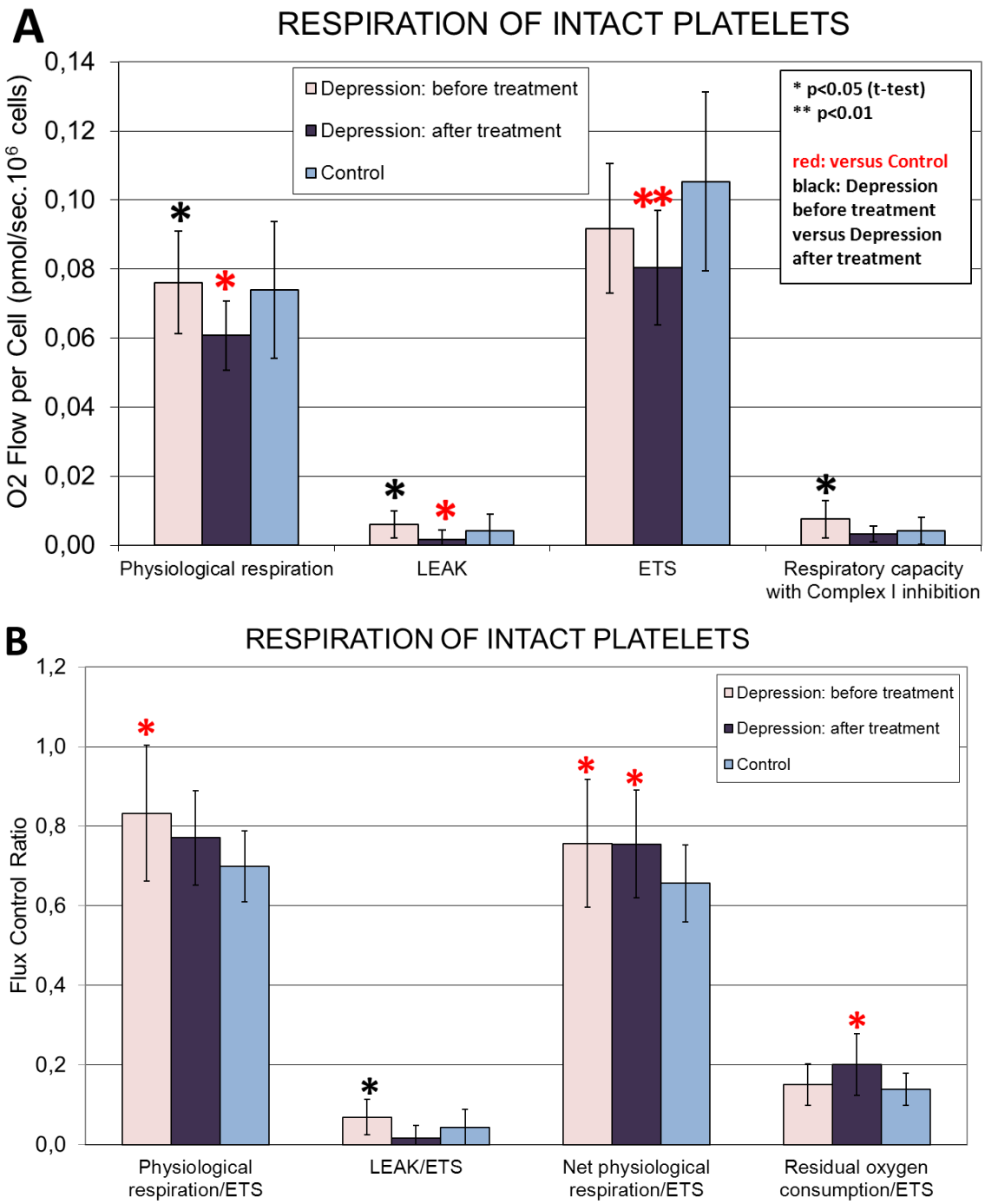

Fig. 13 Mitochondrial respiration in intact platelets. A – O_2 flow per cell, B – Flux Control Ratio (FCR). Platelets were suspended in plasma and/or KH buffer. Samples were measured electrochemically as described in the section "Materials and Methods". Physiological respiration was inhibited by ATPase inhibitor oligomycin and LEAK state (state 4, non-phosphorylating resting state) was induced. The maximal capacity of electron transport system (ETS) was reached by titration of *p*-trifluoromethoxyphenylhydrazone (FCCP). Consequently, complex I was inhibited by rotenone and ETS capacity supported only by complex II. O_2 flows per cell and FCRs of patients were compared to control group and statistical significance was evaluated by the Mann-Whitney U test (*p<0.05).

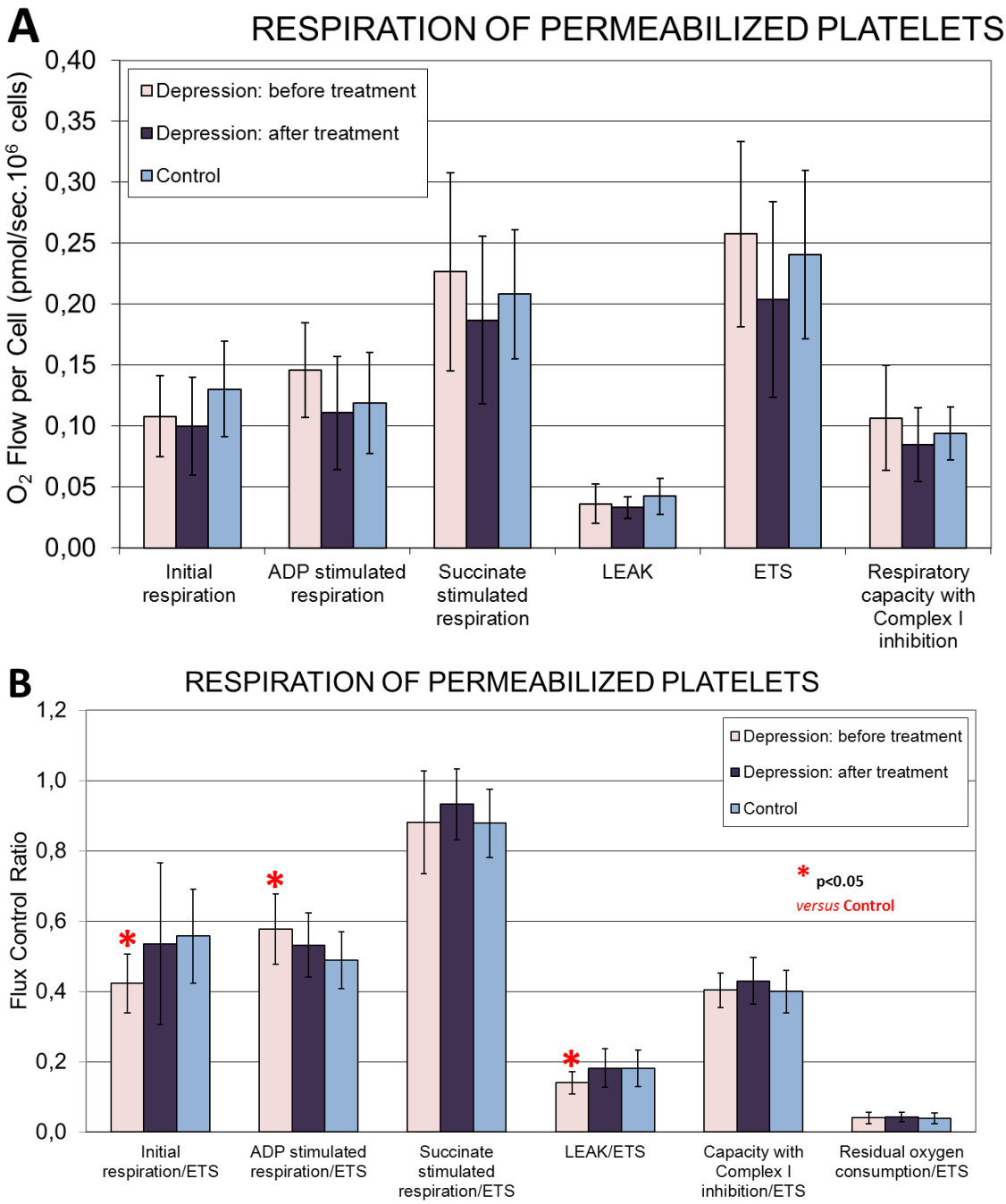

Fig. 14 Mitochondrial respiration in permeabilized cells. A – O_2 flow per cell, B – Flux Control Ratio (FCR). Mitochondrial respiration of platelets resuspended in MiR05 and permeabilized by addition of digitonin. Samples were measured electrochemically as described in the section "Materials and Methods". Initial respiration (state 1) was settled after the stabilization. Substrates of complex I (malate, pyruvate, ADP, glutamate) and complex II (succinate) were added (State 3). OXPHOS was inhibited by addition of oligomycin and LEAK state (state 4) was induced. The maximal capacity of ETS was reached by titration of *p*-trifluoromethoxyphenylhydrazone (FCCP). Consequently, complex I was inhibited by rotenone and ETS capacity supported only by complex II was determined. Folowing addition of antimycine A residual oxygen consumption was measured. O_2 flows per cell and FCRs of patients were compared to control group and statistical significance was evaluated by the Mann-Whitney U test (*$p<0.05$).

5. Discussion

Biological markers of depression and predictors of the antidepressant administration response are searched on the basis of recently known hypotheses of affective disorders, including mitochondrial hypothesis. Leading role of mitochondrial dysfunctions both in pathophysiology of mood disorders and effects of antidepressants was supported by our results.

5.1 Antidepressant effects on mitochondrial functions

Evidences that mitochondrial dysfunctions are included in pathophysiology of psychiatric disorders have been reviewed recently (Shao et al., 2008; Rezin et al., 2009; Jou et al., 2009). They include disturbances in activity of mitochondrial enzymes, impaired calcium signalling and energy metabolism, increased mtDNA deletions, mutations or polymorphisms, and effects of psychotropic drugs on mitochondria. Recent findings provide the evidence that mood-stabilizing drugs are able to prevent dysfunctional mitochondrial ETC-induced oxidative damage (Wang, 2007). However, there is almost no data about direct effects of antidepressants and mood stabilizers on mitochondrial functions; we miss studies comparing effects of pharmacologically different antidepressants on the activities of key enzymes of citric acid cycle and the ETC.

Effects of selected antidepressants and mood stabilizers on mitochondrial functions were examined in pig brain mitochondria. Pig is a relatively unusual species for the most pharmacological studies; however, pig mitochondria are relatively often used in studies of mitochondrial functions. It may be supposed that the pig brain mitochondria are more similar to the human brain mitochondria than rodent mitochondria.

5.1.1 Citric acid cycle enzymes and respiratory chain complexes

Citrate synthase, a rate-limiting enzyme of the TCA cycle, plays a decisive role in regulating energy generation of mitochondrial respiration. In *in vivo* experiments, where venlafaxine was administered chronically, CS activity was not altered. Increased SDH activity was observed in prefrontal cortex, whereas SDH activity was not affected in cerebellum, hippocampus, striatum and cerebral cortex in the brain of rats (Scaini et al., 2010). Drug-induced (by desipramine, amitriptyline, imipramine, citalopram, venlafaxine, tianeptine, lithium, valproate and olanzapine)

increase of citrate synthase activity was found in our experiments. It is consistent with finding that valproate reversed and lithium prevented amphetamine-induced CS inhibition in animal model (Corrêa et al., 2007).

Complex I is a rate limiting enzyme for oxygen consumption in synapses and plays a major role in controlling of OXPHOS (Telford et al., 2009). 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). 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).

5.1.2Respiratory rate

Action of various drugs applied in pharmacotherapy on mitochondria is rather unknown. Some drugs have been specifically designed to affect mitochondrial functions; however, most of them primary act on other cellular targets and may modify mitochondrial functions as adverse effects (Szewczyk and Wojtczak, 2002). Our results demonstrate that pharmacologically different antidepressants (amitriptyline, fluoxetine, tianeptine), but not mood stabilizers (lithium, valproate, olanzapine) can inhibit respiratory rate in mitochondrial preparations from pig brain tissue. Experimental conditions of the present study, use of the selective substrates malate and pyruvate for complex I, and succinate for complex II, allowed the effects of drugs on the respiration activated through complex I and through complex II to be evaluated separately. Hypothesis was confirmed that the pharmacologically different antidepressants or mood stabilizers could act, at least in part, by inhibition of OXPHOS. Thanks to the wide-range of drug

concentration in our titration experiments we were able to calculate matching inhibitory parameters, useful for quantitative comparison of effects of different drugs on mitochondrial respiratory rate.

Inhibitory parameters calculated in this paper result from total concentrations of drugs added to crude brain mitochondrial fraction. Situation in various tissues *in vivo* may be different from our experimental conditions. This was reason why the effects of commonly used antidepressants and mood stabilizers on respiratory rate were compared with effects of chlorpromazine, which is a well-known inhibitor of mitochondrial respiration.

We confirmed and extended previously published results about inhibitory effects of antidepressants of various pharmacological classes on OXPHOS. All tested drugs, except for tianeptine and olanzapine, were more potent inhibitors of respiration activated through complex I compared to respiration through complex II. Fluoxetine as well as amitriptyline seem to be selective inhibitors of respiration through complex I. Tianeptine was found as potent inhibitor of mitochondrial respiration through both complexes. Contrary, ketamine inhibited mitochondrial respiration very weakly or not at all, which can be indicative of different mechanism of its action. All tested mood stabilizers showed only minimal effect on respiratory rate and can be supposed that direct action of these mood stabilizers on OXPHOS activity does not participate on their effects.

In the present study all drugs tested inhibited mitochondrial respiration at higher concentrations. These concentrations were much higher than therapeutically active plasma concentrations of antidepressants *in vivo*. However, most of antidepressants are cationic amphiphilic molecules, which can be accumulated in brain, membranes and subcellular components (Caccia et al., 1990; Karson et al., 1993; Fisar et al., 2004; Fisar et al., 2005; Fisar, 2005). The unique physicochemical properties of the mitochondrial matrix may facilitate the selective accumulation of different xenobiotics in the matrix and/or in the inner mitochondrial membrane (Szewczyk and Wojtczak, 2002). In the presence of membrane potential the distribution of charged lipophilic cations across the membrane will equilibrate with membrane potential leading to the extensive accumulation of the cation in the mitochondrial matrix. There will be a 10-fold accumulation of the cation within mitochondria for every 61.5 mV (at 37 °C) increase in membrane potential. As mitochondrial $\Delta\psi_m$ is typically 140-180 mV, there will be a several hundred-fold accumulation of lipophilic cations into mitochondrial matrix (Ross et al.,

2006). However, according to review of literature, there is no simple correlation between mitochondria targeting capacity and physicochemical properties of drugs. It was concluded that the most common physicochemical factors underlying selective accumulation of xenobiotics within mitochondria are electric potential, ion-trapping, and complex formation with cardiolipin; nonspecific accumulation involves membrane partitioning (Horobin et al., 2007). Thus, high antidepressant concentration can be expected in mitochondria, sufficient for partial inhibition of respiratory rate.

Evidently, sourcing from our results, effect of antidepressants (amitriptyline, fluoxetine, tianeptine) on mitochondrial respiratory rate was inhibitory; effect of mood stabilizers (lithium, valproate, olanzapine) was negligible. Moreover, there are significant differences between several antidepressants, regardless of similar physicochemical properties of their molecules. It indicates the existence of certain selectivity of antidepressant-mitochondria interactions.

Antidepressant-induced decrease of respiratory rate can be associated with adverse effects of pharmacotherapy with antidepressants. However, the possibility is not excluded that antidepressant-induced decrease of respiratory rate is initial event in complex cellular response to antidepressants in 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.

5.1.3Monoamine oxidase activity

Monoamine oxidase is important mitochondrial enzyme that regulates the metabolic degradation of monoamine neurotransmitters in neural and other target tissues. We demonstrated that pharmacologically selective antidepressants can inhibit MAO activity in mitochondrial preparations from brain tissue, modulating the monoaminergic systems in this way. Experimental conditions of the present study, use of the selective substrates 5-HT for MAO-A and PEA for MAO-B, allowed the inhibitory effects and mechanism of the interactions of antidepressants on the two MAO isoforms to be evaluated separately. The potency, isoenzymes selectivity and mechanism of inhibition of representative antidepressants and mood stabilizers towards pig brain MAO were assessed.

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. The reliability of the dissociation constant K_i as an indicator of *in vivo* potency and selectivity is obviously dependent on the accuracy and precision of its *in vitro* estimation. Hypothesis was tested that the pharmacologically selective antidepressants or mood stabilizers could act, at least in part, by inhibition of MAO.

Our study confirmed and extended previous results (Edwards and Burns, 1974; Green and McGachy, 1987; Reid et al., 1988; Egashira et al., 1996, Egashira et al., 1999; Gnerre et al., 2001) that antidepressant drugs of various pharmacological classes (tricyclic antidepressants, SSRI, NRI, SNRI, NaSSA, NDRI, SSRE) show inhibitory effects on MAOs. All tested antidepressants were more potent inhibitors of MAO-A than MAO-B (ratio of IC_{50} for MAO-A to IC_{50} for MAO-B was lower than 0.1 for moclobemide, and varied from 0.45 for mirtazapine to 0.86 for tianeptine). Different IC_{50} or K_i values and mechanisms of inhibition by specific antidepressant were described in various studies; it could be explained both by different experimental conditions and by diverse data evaluation techniques. In accordance with previous studies (Mukherjee and Yang 1997; Mukherjee and Yang, 1999; Gnerre et al., 2001) we found lower affinity of citalopram for MAOs compared to fluoxetine. We observed that olanzapine (antipsychotic and mood stabilizing drug) exhibit similar inhibitory effect on MAOs as antidepressants. Direct *in vitro* inhibitory effect of lithium and valproate was confirmed to be insignificant (Nag, 2004).

We established that moclobemide is a relatively weak MAO-A inhibitor *in vitro*; the dissociation constant of the enzyme-inhibitor complex was found in the range of hundreds of µmol/l (Kettler et al., 1990; Cesura et al., 1992). In spite of its weak MAO-A inhibition *in vitro*, moclobemide is a potent inhibitor of MAO-A *in vivo* (Burkard et al., 1989; Da Prada et al., 1990); plasma concentrations associated with 50% of maximum enzyme activity were found about of one µmol/l (Holford et al., 1994; Guentert et al., 1995). This discrepancy suggested that the compound may be converted *in vivo* to an active form (Kettler et al., 1990). Reversibility of MAO-A inhibition was demonstrated *in vitro* as well as *in vivo* (Haefely et al., 1993). *In vitro* investigations of kinetics of inhibition of MAO-A by moclobemide have shown that inhibition is time-dependent. Initially, the inhibition is competitive, changing gradually to become

noncompetitive (Cesura et al., 1992; Nair et al., 1993). In our experimental conditions moclobemide shown competitive inhibition of MAO-A.

In the present study all drugs tested inhibited MAOs at higher concentrations. These concentrations were much higher than therapeutically active plasma concentrations of antidepressants *in vivo*. However, the brain levels of drugs are a reflection not only of their plasma concentrations, but also of their distribution between plasma and red blood cells (Fišar et al., 1996; Fišar et al., 2006). E.g. the apparent concentration of fluoxetine in brain relative to plasma was found 20:1. The brain concentrations of fluoxetine/norfluoxetine were found to reach to 35 μmol/l (Karson et al., 1993). It has also been shown that fluoxetine is present in subcellular components of the rodent brain, including mitochondria and synaptosomes (Caccia et al., 1990). Thus, with high subcellular concentrations and micromolar affinities for MAO, fluoxetine has the potential to exert a significant inhibitory effect on the enzyme. Consequently, the possibility cannot be excluded that MAO activity is affected due to long lasting action of amphiphilic antidepressants and their accumulation in mitochondrial membrane during chronic treatment.

Since MAO is embedded in the outer mitochondrial membrane, lipid-protein interactions play a role in the functional properties of MAOs (Huang and Faulkner, 1981; Fowler et al., 2007), and antidepressants are capable of changing membrane fluidity, it can be speculated about role of accumulation of antidepressants in the lipid bilayer in their effect on MAO activity. However, it seems that fluidity modulation is not the cause of MAO inhibition (Muriel and Pérez-Rojas, 2003).

The analysis showed that mechanism of inhibition of MAOs by antidepressants may differ with respect both to different drug molecules and to MAO substrates. Competitive inhibition was found for moclobemide and olanzapine only when 5-HT was used as substrate. Competitive inhibition would be consistent with drug binding to the active sites of the MAO-A. Fluoxetine, amitriptyline, citalopram and tianeptine retain the same mechanism of inhibition both for MAO-A and MAO-B.

Inhibitory and kinetic parameters calculated in this paper result from total concentrations of drugs added to crude brain mitochondrial fraction. Situation in various tissues *in vivo* may be different from our experimental conditions. This was reason why the effects of commonly used antidepressants and mood stabilizers on MAOs activity were compared with effects of well-known MAO inhibitors such as iproniazid, pargyline and clorgyline. The results shown that all

tested antidepressants are much weaker MAOs inhibitors compared to these irreversible MAOIs. It was suggested that inhibition of brain MAO could contribute to slowing, halting and maybe reversing of neurodegeneration (e.g. in dopaminergic neurons) which was initiated by oxidative stress (Youdim et al., 2006). Neuroprotection has been uncovered as common effect of long-term treatment of mood disorders and Parkinson's disease with different antidepressants, mood stabilizers and antipsychotics. The molecular mechanism of neuroprotection is not yet clear; however, it is obvious that reduction of local oxidative stress is a major component, and MAO inhibition participates in this reduction.

An unanswered question in the field pharmacotherapy of mental disorders is why some individuals are more vulnerable than others to substance use disorders or to therapeutic effects of psychotropic drugs. Differences both in drug metabolism and in activity of neurotransmitter systems are included in interindividual differences in response to drug treatment. Antidepressants and numerous other widely used drugs also are unlabeled MAO inhibitors. Exact physiological role of MAO inhibition by antidepressants tested (except for moclobemide) is not known, however, the effects of drugs on MAO activity may contribute to their modulation of monoamine neurotransmission in the brain. It can be speculated that inhibition of MAO by pharmacologically different antidepressants could contribute to the regulation of mood and emotions or to the interindividual differences in the drug response.

In general, pharmacologically different antidepressants were found to be rather weak MAO inhibitors. There is evident discrimination both between several drugs and the two MAO isoforms. Because *in vitro* inhibition of MAOs by antidepressants was demonstrated and there is drug accumulation in the brain tissue, hypothesis was supported that *in vivo* inhibition of MAO by pharmacologically selective antidepressants would amplify their effects on serotonergic, noradrenergic and dopaminergic neurotransmission. Although it is always difficult to extrapolate *in vitro* studies to the clinical reality, the present results suggest that inhibitory effect of antidepressants on MAO activity cannot be neglected.

5.2 Mitochondrial respiration in platelets of depressive subjects

We examined temporal changes of oxygen consumption in blood platelets of depressive patients before the treatment, after the treatment, and in controls. Effects of psychotropic drugs on mitochondria contribute to their role in psychiatric disorders (Hroudová and Fišar, 2011;

Hroudova and Fisar, 2010). Reversely, psychiatric symptoms often preceded the diagnosis of mitochondrial disease (Fattal et al., 2006; Fattal et al., 2007). The result of impaired mitochondrial functions is complex and includes increased generation of reactive oxygen and nitrogen species, decreased antioxidant levels and changes of balance between antiapoptotic and apoptotic factors (Calabrese et al., 2000). Both disturbed production and detoxification of reactive oxygen and nitrogen species participate on physiological effects of mitochondrial dysfunctions (Starkov 2008; Maes et al., 2012; Horan et al., 2012).

Platelets are an easy available source of mitochondria and were proved to be suitable for analysis of mitochondrial respiration. Energy metabolism of human platelets is composed of oxidative phosphorylation and glycolysis, both energy pathways are necessary for normal function of platelets. Interrelationship between these two pathways is mediated by ADP/ATP ratios (Doery et al., 1970). Mitochondrial functions were assessed both in intact and permeabilized blood platelets. Measurement of respiration during action of appropriate endogenous and exogenous substances enables the identification of the primary sites of effectors and the distribution of control, allowing deeper quantitative analyses (Brand and Nichols, 2011). Addition of different substrates is important to stimulate the flow of electrons from ETC complexes and allows the identification of specific malfunctioning complexes. Assessments of cellular respiration were performed immediately after the blood taking, as ATP synthase and complex IV activities can be declined during storage (Diab et al., 2012). Evaluation of oxygen flux was expressed by two parameters: 1. oxygen flow per cells, a system-specific quantity relative to blood platelets; and 2. flux control ratio (FCR) calculated as ratio of respiratory rate at a reference state to maximal respiratory capacity of ETS.

In our preliminary experiments with intact platelets, both physiological mitochondrial respiration and maximal capacity of ETS 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. Measurements in permeabilized platelets support these suggestions.

Recent 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 side effects of pharmacotherapy. We propose that changes in oxygen consumption may participate in pathophysiology of depression. Increased FCR in depressive subjects might be a result of the depressive disorder as well as treatment with antidepressants; our data indicates determining role of antidepressant-induced decrease of mitochondrial respiratory capacity. Better insight into molecular mechanisms of cellular respiration and control of OXPHOS is likely to lead to better understanding of pathophysiology of psychiatric disorders or interindividual variations in response to pharmacotherapy. However, a systematic *in vivo* investigation of the antidepressants or mood stabilizers effects on respiratory rate is necessary to confirm the clinically important conclusion.

6. Conclusions

Biological markers of depression, predictors of the response to the drug administration and molecular targets of new antidepressants are searched on the basis of recently known hypotheses of affective disorders. We come out mostly from stimuli of neurotrophic hypothesis and mitochondrial hypothesis. According to these hypotheses, the leading role in the pathophysiology of mood disorders and therapeutic effects of antidepressants have mitochondria, which are destined for changes in energetic metabolism of cells. Mitochondrial dysfunctions and thereby impaired neuronal metabolism can lead to disturbances in neuronal function, plasticity and brain circuitry. Impaired functions of mitochondria contribute to a wide range of diseases; the role of mitochondria in the pathophysiology of schizophrenia, bipolar disorder, and major depressive disorder is supported by studies investigating genomic differences, changes of energy metabolism and mitochondrial changes included. Variations in the mitochondrial genome as well as defects of ETC have been implicated in the pathogenesis of psychiatric diseases.

We confirmed the presumption that interactions between psychopharmaca and OXPHOS can significantly influence intracellular processes related to psychiatric disorders. Supposing that the mechanism of action of antidepressants and mood stabilizers is related to processes implicated in pathophysiology of mood disorders, our results designate complexes I and IV of respiratory ETC both as targets of these drugs and as components modified during the illness. These mitochondrial enzymes are suggested as proper candidates in searching for new biological markers of mood disorders, targets of new antidepressants or predictors of response to pharmacotherapy.

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. Variability in intracellular processes probably participates in the interindividual differences of the response to treatment with antidepressant or in drug resistance. 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 effects or to side effects of pharmacotherapy.

In vitro results comprised marked drug-induced changes of mitochondrial enzymes activities. Differences in inhibitory potency and in mechanism of inhibition have been found between several drugs. Antidepressants, but not mood stabilizers, seem to be potent partial inhibitors of

mitochondrial respiration supported both complex I and complex II substrates. MAOs, citrate synthase and complexes I and IV of ECT were the most affected mitochondrial enzymes and they can be suggested as proper candidates for biological markers of mood disorders, targets of new pharmaca or predictors of response to pharmacotherapy. Effect of newly synthetized psychotropic drugs on mitochondrial respiration should be included in their testing to discover their mitochondrial toxicity and/or potential neurotrophic effects; high-resolution respirometry is suitable sensitive technique for these measurements. Multiple substrate-uncoupler-inhibitor titration protocols for high-resolution respirometry are useful for sensitive and accurate measurement of respiratory rate in both intact and permeabilized human platelets. Mitochondrial respiration is affected by antidepressants significantly more than by depressive disorder in itself. Relationships of antidepressant-induced changes in cellular respiration to adverse or therapeutic effects of pharmacotherapy need to be discovered.

Results from our measurements and analyses revealed how far mitochondrial functions are sensitive to effects of antidepressants and mood disorders. The methodology of *in vitro* measurement has been consequently modified to measure the same mitochondrial parameters (respiratory rate and mitochondrial enzyme activities) in blood platelets of depressive patients. Analysis of these data enabled us to determine suitable mitochondrial parameters, which are measurable in peripheral blood. Acceptable parameters could be further studied as potential biological markers of the disorder, in order to get them in early diagnostics of depressive disorder, prediction of efficacy or occurrence adverse effects of pharmacotherapy, and possibly use them as intracellular targets of newly developed antidepressants.

References

1. aan het Rot M, Mathew SJ, Charney DS. Neurobiological mechanisms in major depressive disorder. CMAJ. 2009; 180(3): 305-313.
2. Abdel-Razaq W, Kendall DA, Bates TE. The effects of antidepressants on mitochondrial function in a model cell system and isolated mitochondria. Neurochem Res. 2011; 36(2): 327-338.
3. Agostinho FR, Réus GZ, Stringari RB, Ribeiro KF, Ferraro AK, Benedet J, Rochi N, Scaini G, Streck EL, Quevedo J. Treatment with olanzapine, fluoxetine and olanzapine/fluoxetine alters citrate synthase activity in rat brain. Neurosci Lett. 2011; 487(3): 278-281.
4. Aires CC, Soveral G, Luís PB, ten Brink HJ, de Almeida IT, Duran M, Wanders RJ, Silva MF. Pyruvate uptake is inhibited by valproic acid and metabolites in mitochondrial membranes. FEBS Lett. 2008; 582(23-24): 3359-3366.
5. Akaike H. A new look at the statistical model identification. IEEE Transactions on Automatic Control. 1974; 19(6): 716-723.
6. Arnold S, Goglia F, Kadenbach B. 3,5-Diiodothyronine binds to subunit Va of cytochrome-c oxidase and abolishes the allosteric inhibition of respiration by ATP. Eur J Biochem. 1998; 252(2): 325-330.
7. Arnold S, Kadenbach B. Cell respiration is controlled by ATP, an allosteric inhibitor of cytochrome-c oxidase. Eur J Biochem. 1997; 249(1): 350-354.
8. Aronis A, Melendez JA, Golan O, Shilo S, Dicter N, Tirosh O. Potentiation of Fas-mediated apoptosis by attenuated production of mitochondria-derived reactive oxygen species. Cell Death Differ. 2003; 10(3): 335-344.
9. Atlante A, Calissano P, Bobba A, Giannattasio S, Marra E, Passarella S. Glutamate neurotoxicity, oxidative stress and mitochondria. FEBS Lett. 2001; 497(1): 1-5.
10. Azzu V, Jastroch M, Divakaruni AS, Brand MD. The regulation and turnover of mitochondrial uncoupling proteins. Biochim Biophys Acta. 2010; 1797(6-7): 785-791.
11. Bach AWJ, Lan NC, Johnson DL, Abell CW, Bembenek ME, Kwan S-W, Seeburg PH, Shih JC. cDNA cloning of human liver monoamine oxidase A and B: molecular basis of differences in enzymatic properties. Proc Natl Acad Sci U S A. 1988; 85(13): 4934-4938.
12. Bachmann RF, Wang Y, Yuan P, Zhou R, Li X, Alesci S, Du J, Manji HK. Common effects of lithium and valproate on mitochondrial functions: protection against

methamphetamine-induced mitochondrial damage. Int J Neuropsychopharmacol. 2009; 12(6): 805-822.

13. Baines CP, Kaiser RA, Sheiko T, Craigen WJ, Molkentin JD. Voltage-dependent anion channels are dispensable for mitochondrial-dependent cell death. Nat Cell Biol. 2007; 9(5): 550-555.
14. Baker GB, Urichuk LJ, McKenna KF, Kennedy SH. Metabolism of monoamine oxidase inhibitors. Cell Mol Neurobiol. 1999; 19(3): 411-426.
15. Balon R. Biological predictors of antidepressant treatment outcome. Clin Neuropharmacol. 1989; 12(3): 195-214.
16. Beck V, Jabůrek M, Demina T, Rupprecht A, Porter RK, Jezek P, Pohl EE. Polyunsaturated fatty acids activate human uncoupling proteins 1 and 2 in planar lipid bilayers. FASEB J. 2007; 21(4): 1137-1144.
17. Becker CM, Harris RA. Influence of valproic acid on hepatic carbohydrate and lipid metabolism. Arch Biochem Biophys. 1983; 223(2): 381-392.
18. Benard G, Bellance N, Jose C, Melser S, Nouette-Gaulain K, Rossignol R. Multi-site control and regulation of mitochondrial energy production. Biochim Biophys Acta. 2010; 1797(6-7): 698-709.
19. Bender E, Kadenbach B. The allosteric ATP-inhibition of cytochrome c oxidase activity is reversibly switched on by cAMP-dependent phosphorylation. FEBS Lett. 2000; 466(1):130-134.
20. Ben-Shachar D, Karry R. Neuroanatomical pattern of mitochondrial complex I pathology varies between schizophrenia, bipolar disorder and major depression. PLoS ONE. 2008; 3(11): e3676.
21. Berger I, Segal I, Shmueli D, Saada A. The effect of antiepileptic drugs on mitochondrial activity: a pilot study. J Child Neurol. 2010; 25(5): 541-545.
22. Berman RM, Sanacora G, Anand A, Roach LM, Fasula MK, Finkelstein CO, Wachen RM, Oren DA, Heninger GR, Charney DS. Monoamine depletion in unmedicated depressed subjects. Biol Psychiatry. 2002; 51(6): 469-473.
23. Boffoli D, Scacco SC, Vergari R, Solarino G, Santacroce G, Papa S. Decline with age of the respiratory chain activity in human skeletal muscle. Biochim Biophys Acta. 1994; 1226(1): 73-82.

24. Bolaños JP, Almeida A, Moncada S. Glycolysis: a bioenergetic or a survival pathway? Trends Biochem Sci. 2010; 35(3): 145-149.
25. Bolaños JP, Moro MA, Lizasoain I, Almeida A. Mitochondria and reactive oxygen and nitrogen species in neurological disorders and stroke: Therapeutic implications. Adv Drug Deliv Rev. 2009; 61(14): 1299-1315.
26. Bonnet U. Moclobemide: therapeutic use and clinical studies. CNS Drug Rev. 2003; 9(1): 97-140.
27. Boss O, Hagen T, Lowell BB. Uncoupling proteins 2 and 3: potential regulators of mitochondrial energy metabolism. Diabetes. 2000; 49(2): 143-156.
28. Bown CD, Wang JF, Young LT. Attenuation of N-methyl-D-aspartate-mediated cytoplasmic vacuolization in primary rat hippocampal neurons by mood stabilizers. Neuroscience. 2003; 117(4): 949-955.
29. Bown CD, Wang JF, Chen B, Young LT. Regulation of ER stress proteins by valproate: therapeutic implications. Bipolar Disord. 2002; 4(2): 145-151.
30. Brand MD, Affourtit C, Esteves TC, Green K, Lambert AJ, Miwa S, Pakay JL, Parker N. Mitochondrial superoxide: production, biological effects, and activation of uncoupling proteins. Free Radic Biol Med. 2004; 37(6): 755-767.
31. Brand MD, Nicholls DG. Assessing mitochondrial dysfunction in cells. Biochem J. 2011; 435(2): 297-312.
32. Brand MD, Pakay JL, Ocloo A, Kokoszka J, Wallace DC, Brookes PS, Cornwall EJ. The basal proton conductance of mitochondria depends on adenine nucleotide translocase content. Biochem J. 2005; 392(Pt 2): 353-362.
33. Brand MD, Turner N, Ocloo A, Else PL, Hulbert AJ. Proton conductance and fatty acyl composition of liver mitochondria correlates with body mass in birds. Biochem J. 2003; 376(Pt 3): 741-748.
34. Bunoust O, Devin A, Avéret N, Camougrand N, Rigoulet M. Competition of electrons to enter the respiratory chain: a new regulatory mechanism of oxidative metabolism in Saccharomyces cerevisiae.J Biol Chem. 2005; 280(5): 3407-3413.
35. Burbenskaya NM, Nartsissov YR, Tsofina LM, Komissarova IA. The uncoupling effect of some psychotropic drugs on oxidative phosphorylation in rat liver mitochondria. Biochem Mol Biol Int. 1998; 45(2): 261-268.

36. Burkard WP, Da Prada M, Keller HH, Kettler R, Haefely W. Pre-clinical pharmacology of moclobemide. A review of published studies. Br J Psychiatry Suppl. 1989; (6): 84-88.
37. Caccia S, Cappi M, Fracasso C, Garattini S.: Influence of dose and route of administration on the kinetics of fluoxetine and its metabolite norfluoxetine in the rat. Psychopharmacology (Berl). 1990; 100(4): 509-514.
38. Cadenas E, Davies KL. Mitochondrial free radical generation, oxidative stress, and aging. Free Radic Biol Med. 2000; 29: 222-230.
39. Cai Q, Sheng ZH. Mitochondrial transport and docking in axons. Exp Neurol. 2009; 218(2): 257-267.
40. Cairns CB, Walther J, Harken AH, Banerjee A. Mitochondrial oxidative phosphorylation thermodynamic efficiencies reflect physiological organ roles. Am J Physiol. 1998; 274(5 Pt 2): R1376-R1383.
41. Calabrese V, Bates TE, Stella AM. NO synthase and NO-dependentsignalpathways in brainaging and neurodegenerativedisorders: the role of oxidant/antioxidantbalance. Neurochem Res. 2000; 25(9-10): 1315-1341.
42. Calabrese V, Cornelius C, Dinkova-Kostova AT, Calabrese EJ, Mattson MP. Cellular stress responses, the hormesis paradigm, and vitagenes: novel targets for therapeutic intervention in neurodegenerative disorders. Antioxid Redox Signal. 2010; 13(11): 1763-1811.
43. Cannino G, Di Liegro CM, Rinaldi AM. Nuclear-mitochondrial interaction. Mitochondrion. 2007; 7(6): 359-366.
44. Catena-Dell'Osso M, Bellantuono C, Consoli G, Baroni S, Rotella F, Marazziti D. Inflammatory and neurodegenerative pathways in depression: a new avenue for antidepressant development? Curr Med Chem. 2011; 18(2): 245-255.
45. Cesura AM, Kettler R, Imhof R, Da Prada M. Mode of action and characteristics of monoamine oxidase-A inhibition by moclobemide. Psychopharmacology (Berl). 1992; 106 Suppl: S15-S16.
46. Chada SR, Hollenberck PJ. Nerve growth factor signaling regulates motility and docking of axonal mitochondria. Curr Biol. 2004; 14(14): 1272-1276.
47. Chance B, Willams GR. The respiratory chain and oxidative phosphorylation. Adv Enzymol Relat Subj Biochem. 1956; 17: 65-134.

48. Chance B, Williams GR. Respiratory enzymes in oxidative phosphorylation. III. The steady state. J Biol Chem. 1955; 217(1): 409-427.
49. Chang DT, Reynolds IJ. Differences in mitochondrial movement and morphology in young and mature primary cortical neurons in culture. Neuroscience. 2006; 141(2): 727-736.
50. Chen G, Pan B, Hawver DB, Wright CB, Potter WZ, Manji HK. Attenuation of cyclic AMP production by carbamazepine. J Neurochem. 1996; 67(5): 2079-2086.
51. Chen G, Zeng WZ, Yuan PX, Huang LD, Jiang YM, Zhao ZH, Manji HK. The mood-stabilizing agents lithium and valproate robustly increase the levels of the neuroprotective protein bcl-2 in the CNS. J Neurochem. 1999; 72(2): 879-882.
52. Chen K, Wu HF, Shih JC. The deduced amino acid sequences of human platelet and frontal cortex monoamine oxidase B are identical. J Neurochem. 1993; 61(1): 187-190.
53. Chen Q, Vazquez EJ, Moghaddas S, Hoppel CL, Lesnefsky EJ. Production of reactive oxygen species by mitochondria. Central role of complex III. J Biol Chem. 2003; 278(38): 36027-36031.
54. Chen Y, McMillan-Ward E, Kong J, Israels SJ, Gibson SB. Mitochondrial electron-transport-chain inhibitors of complexes I and II induce autophagic cell death mediated by reactive oxygen species. J Cell Sci. 2007; 120(Pt 23): 4155-4166.
55. Cheng SY, Leonard JL, Davis PJ. Molecular aspects of thyroid hormone actions. Endocr Rev. 2010; 31(2): 139-170.
56. Cheng Y. Prusoff WH. Relationship between the inhibition constant (K_1) and the concentration of inhibitor which causes 50 per cent inhibition (I_{50}) of an enzymatic reaction. Biochem Pharmacol. 1973; 22(23): 3099-3108.
57. Chuang DM. The antiapoptotic actions of mood stabilizers: molecular mechanisms and therapeutic potentials. Ann N Y Acad Sci. 2005; 1053: 195-204.
58. Cocco T, Pacelli C, Sgobbo P, Villani G. Control of OXPHOS efficiency by complex I in brain mitochondria. Neurobiol Aging. 2009; 30(4): 622-629.
59. Coppen A. The biochemistry of affective disorders. Br J Psychiatry. 1967; 113(504): 1237-1264.
60. Corrêa C, Amboni G, Assis LC, Martins MR, Kapczinski F, Streck EL, Quevedo J. Effects of lithium and valproate on hippocampus citrate synthase activity in an animal model of mania. Prog Neuropsychopharmacol Biol Psychiatry. 2007; 31(4): 887-891.

61. Costa C, Belcastro V, Tozzi A, Di Filippo M, Tantucci M, Siliquini S, Autuori A, Picconi B, Spillantini MG, Fedele E, Pittaluga A, Raiteri M, Calabresi P. Electrophysiology and pharmacology of striatal neuronal dysfunction induced by mitochondrial complex I inhibition. J Neurosci. 2008; 28(32): 8040-8052.
62. Cowen PJ.Serotonin and depression: pathophysiological mechanism or marketing myth? Trends Pharmacol Sci. 2008; 29(9): 433-436.
63. Curti C, Mingatto FE, Polizello AC, Galastri LO, Uyemura SA, Santos AC. Fluoxetine interacts with the lipid bilayer of the inner membrane in isolated rat brain mitochondria, inhibiting electron transport and F1F0-ATPase activity. Mol Cell Biochem. 1999; 199(1-2): 103-109.
64. Da Prada M, Cesura AM, Launay JM, Richards JG. Platelets as a model for neurones? Experientia. 1988; 44(2): 115-126.
65. Da Prada M, Kettler R, Keller HH, Burkard WP, Muggli-Maniglio D, Haefely WE. Neurochemical profile of moclobemide, a short-acting and reversible inhibitor of monoamine oxidase type A. J Pharmacol Exp Ther. 1989; 248(1): 400-414.
66. Da Prada M, Kettler R, Keller HH, Cesura AM, Richards JG, Saura Marti J, Muggli-Maniglio D, Wyss PC, Kyburz E, Imhof R. From moclobemide to Ro 19-6327 and Ro 41-1049: the development of a new class of reversible, selective MAO-A and MAO-B inhibitors. J Neural Transm Suppl. 1990; 29: 279-292.
67. Dager SR, Friedman SD, Parow A, Demopulos C, Stoll AL, Lyoo IK, Dunner DL, Renshaw PF. Brain metabolic alterations in medication-free patients with bipolar disorder. Arch Gen Psychiatry. 2004; 61(5): 450-458.
68. Davey GP, Peuchen S, Clark JB. Energy thresholds in brain mitochondria: potential involvement in neurodegeneration. J. Biol. Chem. 1998; 273: 12753-12757.
69. Davidson J, Mcleod MN, Linnoila M, Kurland AA, White HL. Platelet MAO inhibition following tricyclic antidepressant therapy. Am J Psychiatry. 1978; 135(5): 603-605.
70. De Marchi U, Castelbou C, Demaurex N. Uncoupling protein 3 (UCP3) modulates the activity of Sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) by decreasing mitochondrial ATP production. J Biol Chem. 2011; 286(37): 32533-32541.
71. de Vasconcellos AP, Zugno AI, Dos Santos AH, Nietto FB, Crema LM, Gonçalves M, Franzon R, de Souza Wyse AT, da Rocha ER, Dalmaz C. Na+,K(+)-ATPase activity is

reduced in hippocampus of rats submitted to an experimental model of depression: effect of chronic lithium treatment and possible involvement in learning deficits. Neurobiol Learn Mem. 2005; 84(2): 102-110.

72. Delgado PL, Miller HL, Salomon RM, Licinio J, Krystal JH, Moreno FA, Heninger GR, Charney DS. Tryptophan-depletion challenge in depressed patients treated with desipramine or fluoxetine: implications for the role of serotonin in the mechanism of antidepressant action. Biol Psychiatry. 1999; 46(2): 212-220.
73. Diab YA, Thomas A, Luban NL, Wong EC, Wagner SJ, Levy RJ. Acquired cytochrome C oxidase impairment in apheresis platelets during storage: a possible mechanism for depletion of metabolic adenosine triphosphate. Transfusion. 2012; 52(5): 1024-1030.
74. Di Paola M, Lorusso M. Interaction of free fatty acids with mitochondria: coupling, uncoupling and permeability transition. Biochim Biophys Acta. 2006; 1757(9-10): 1330-1337.
75. Doery JC, Hirsh J, Cooper I. Energy metabolism in human platelets: interrelationship between glycolysis and oxidative metabolism. Blood. 1970; 36(2): 159-168.
76. Douette P, Sluse FE. Mitochondrial uncoupling proteins: new insights from functional and proteomic studies. Free Radic Biol Med. 2006; 40(7): 1097-1107.
77. Duman RS, Heninger GR, Nestler, EJ. A molecular and cellular theory of depression. Arch Gen Psychiatry. 1997; 54(7): 597-606.
78. Duman RS. Depression: a case of neuronal life and death? Biol Psychiatry. 2004; 56(3): 140-145.
79. Duman RS. Synaptic plasticity and mood disorders. Mol Psychiatry. 2002; 7(Suppl 1): S29-S34.
80. Dykens JA, Jamieson JD, Marroquin LD, Nadanaciva S, Xu JJ, Dunn MC, Smith AR, Will Y. In vitro assessment of mitochondrial dysfunction and cytotoxicity of nefazodone, trazodone, and buspirone. Toxicol Sci. 2008; 103(2): 335-345.
81. Echtay KS, Roussel D, St-Pierre J, Jekabsons MB, Cadenas S, Stuart JA, Harper JA, Roebuck SJ, Morrison A, Pickering S, Clapham JC, Brand MD. Superoxide activates mitochondrial uncoupling proteins. Nature. 2002; 415(6867): 96-99.
82. Edwards DJ, Burns MO. Effects of tricyclic antidepressants upon human platelet monoamine oxidase. Life Sci. 1974; 15(12): 2045-2058.

83. Egashira T, Kuroiwa Y, Kamijo K. Multiple catalytic sites of rat brain mitochondrial monoamine oxidase. Arch Biochem Biophys. 1978; 191(2): 714-718.
84. Egashira T, Takayama F, Yamanaka Y. Effects of long-term treatment with dicyclic, tricyclic, tetracyclic, and noncyclic antidepressant drugs on monoamine oxidase activity in mouse brain. Gen Pharmacol. 1996; 27(5): 773-778.
85. Egashira T, Takayama F, Yamanaka Y. The inhibition of monoamine oxidase activity by various antidepressants: differences found in various mammalian species. Jpn J Pharmacol. 1999; 81(1): 115-121.
86. Einat, H, Manji HK. Cellular plasticity cascades: genes-to-behavior pathways in animal models of bipolar disorder. Biol Psychiatry. 2006; 59(12): 1160-1171.
87. Ekstedt B. Substrate specificity of the different forms of monoamine oxidase in rat liver mitochondria. Biochem Pharmacol. 1976; 25(10): 1133-1138.
88. Erecinska M, Cherian S, Silver IA. Energy metabolism in mammalian brain during development. Prog Neurobiol. 2004; 73(6): 397-445.
89. Fagervall I, Ross SB. Inhibition of monoamine oxidase in monoaminergic neurones in the rat brain by irreversible inhibitors. Biochem Pharmacol. 1986; 35(8): 1381-1387.
90. Fattal O, Budur K, Vaughan AJ, Franco K. Review of the literature on major mental disorders in adult patients with mitochondrial diseases. Psychosomatics. 2006; 47(1): 1-7.
91. Fattal O, Link J, Quinn K, Cohen BH, Franco K. Psychiatric comorbidity in 36 adults with mitochondrial cytopathies. CNS Spectr. 2007; 12(6): 429-38.
92. Ferguson M, Mockett RJ, Shen Y, Orr WC, Sohal RS. Age-associated decline in mitochondrial respiration and electron transport in Drosophila melanogaster. Biochem J. 2005; 390(Pt 2): 501-511.
93. Fisar Z, Fuksová K, Velenovská M. Binding of imipramine to phospholipid bilayers using radioligand binding assay. Gen Physiol Biophys. 2004; 23(1): 77-99.
94. Fisar Z, Anders M, Tvrzická E, Stanková B. Effect of long-term administration of antidepressants on the lipid composition of brain plasma membranes. Gen Physiol Biophys. 2005; 24(2): 221-236.
95. Fisar Z. Interactions between tricyclic antidepressants and phospholipid bilayer membranes. Gen Physiol Biophys. 2005; 24(2): 161-180.

96. Fišar Z, Fuksová K, Sikora J, Kališová L, Velenovská M, Novotná M. Distribution of antidepressants between plasma and red blood cells. Neuro Endocrinol Lett. 2006; 27(3): 307-313.
97. Fišar Z, Hroudová J, Raboch J. Inhibition of monoamine oxidase activity by antidepressants and mood stabilizers. Neuro Endocrinol Lett. 2010; 31(5): 645-656.
98. Fišar Z, Hroudová J. Common aspects of neuroplasticity, mood disorders and mitochondrial functions. Act Nerv Super Rediviva. 2010a; 52(1): 3-20.
99. Fišar Z, Hroudová J. Intracellular signalling pathways and mood disorders. Folia Biol. 2010b; 56(4): 135-148.
100. Fišar Z, Jirák R, Bob P, Papežová H. Vybrané kapitoly z biologické psychiatrie, 2. přepracované a doplněné vydání. Grada Publishing, a.s., Praha; 2009.
101. Fišar Z, Krulík R, Fuksová K, Sikora J. Imipramine distribution among red blood cells, plasma and brain tissue. Gen Physiol Biophys. 1996; 15(1): 51-64.
102. Fišar Z, Raboch J. Depression, antidepressants, and peripheral blood components. Neuro Endocrinol Lett. 2008; 29(1): 17-28.
103. Fišar Z. Biochemické hypotézy afektivních poruch. Galén, Praha; 1998.
104. Fišar Z. Cannabinoids and monoamine neurotransmission with focus on monoamine oxidase. Prog Neuropsychopharmacol Biol Psychiatry. 2011 Dec 30. [Epub ahead of print]
105. Fisar Z. Inhibition of monoamine oxidase activity by cannabinoids. Naunyn Schmiedebergs Arch Pharmacol. 2010; 381(6): 563-572.
106. Fischer JC, Ruitenbeek W, Stadhouders AM, Trijbels JM, Sengers RC, Janssen AJ, Veerkamp JH. Investigation of mitochondrial metabolism in small human skeletal muscle biopsy specimens. Improvement of preparation procedure. Clin Chim Acta. 1985; 145(1): 89-99.
107. Folbergrová J, Ješina P, Drahota Z, Lisý V, Haugvicová R, Vojtíšková A, Houstěk J. Mitochondrial complex I inhibition in cerebral cortex of immature rats following homocysteic acid-induced seizures. Exp Neurol. 2007; 204(2): 597-609.
108. Foster KA, Galeffi F, Gerich FJ, Turner DA, Müller M. Optical and pharmacological tools to investigate the role of mitochondria during oxidative stress and neurodegeneration. Prog Neurobiol. 2006; 79(3): 136-171.

109. Fowler CJ, Tipton KF. Concentration dependence of the oxidation of tyramine by the two forms of rat liver mitochondrial monoamine oxidase. Biochem Pharmacol. 1981; 30(24): 3329-3332.
110. Fowler PW, Balali-Mood K, Deol S, Coveney PV, Sansom MS. Monotopic enzymes and lipid bilayers: a comparative study. Biochemistry. 2007; 46(11): 3108-3115.
111. Frey BN, Andreazza AC, Nery FG, Martins MR, Quevedo J, Soares JC, Kapczinski F. The role of hippocampus in the pathophysiology of bipolar disorder. Behav Pharmacol. 2007a; 18(5-6): 419-430.
112. Frey BN, Stanley JA, Nery FG, Monkul ES, Nicoletti MA, Chen HH, Hatch JP, Caetano SC, Ortiz O, Kapczinski F, Soares JC. Abnormal cellular energy and phospholipid metabolism in the left dorsolateral prefrontal cortex of medication-free individuals with bipolar disorder: an in vivo ^{1}H MRS study. Bipolar Disord. 2007b; 9(S1): 119-127.
113. Fromenty B, Freneaux E, Labbe G, Deschamps D, Larrey D, Letteron P, Pessayre D. Tianeptine, a new tricyclic antidepressant metabolized by beta-oxidation of its heptanoic side chain, inhibits the mitochondrial oxidation of medium and short chain fatty acids in mice. Biochem Pharmacol. 1989; 38(21): 3743-3751.
114. Gamaro GD, Streck EL, Matté C, Prediger ME, Wyse AT, Dalmaz C. Reduction of hippocampal Na+, K+-ATPase activity in rats subjected to an experimental model of depression. Neurochem Res. 2003; 28(9): 1339-1344.
115. Gnaiger E. Bioenergetics at low oxygen: dependence of respiration and phosphorylation on oxygen and adenosine diphosphate supply. Respir Physiol. 2001; 128(3): 277-297.
116. Gnaiger E. Capacity of oxidative phosphorylation in human skeletal muscle: new perspectives of mitochondrial physiology.Int J Biochem Cell Biol. 2009; 41(10): 1837-1845.
117. Gnerre C, Kosel M, Baumann P, Carrupt PA, Testa B. Interaction of psychotropic drugs with monoamine oxidase in rat brain. J Pharm Pharmacol. 2001; 53(8): 1125-1130.
118. Goglia F, Moreno M, Lanni A. Action of thyroid hormones at the cellular level: the mitochondrial target. FEBS Lett. 1999; 452(3): 115-120.
119. Gould TD, Manji HK. Glycogen synthase kinase-3: a putative molecular target for lithium mimetic drugs. Neuropsychopharmacology. 2005; 30(7): 1223-1237.

120. Gould TD, Quiroz JA, Singh J, Zarate CA, Manji HK. Emerging experimental therapeutics for bipolar disorder: insights from the molecular and cellular actions of current mood stabilizers. Mol Psychiatry. 2004; 9(8): 734-755.
121. Green AL, McGachy HA. The inhibition of monoamine oxidase by tricyclic antidepressants: the influence of the nature of the substrate and the source of the enzyme. J Pharm Pharmacol. 1987; 39(5): 392-394.
122. Green AL, O'Grady JE, Vass M. The effect of some tricyclic antidepressants on the inhibition of mouse brain monoamine oxidase in-vivo by phenelzine. J Pharm Pharmacol. 1989; 41(1): 50-51.
123. Guentert TW, Banken L, Hilton S, Holford NH. Moclobemide: relationships between dose, drug concentration in plasma, and occurrence of adverse events. J Clin Psychopharmacol. 1995; 15(4 Suppl 2): 84S-94S.
124. Haas R, Stumpf DA, Parks JK, Eguren L. Inhibitory effects of sodium valproate on oxidative phosphorylation. Neurology. 1981; 31(11): 1473-1476.
125. Haddad JJ, Saadé NE, Safieh-Garabedian B. Cytokines and neuro-immune-endocrine interactions: a role for the hypothalamic-pituitary-adrenal revolving axis. J Neuroimmunol. 2002; 133(1-2): 1-19.
126. Haddjeri N, Szabo ST, de Montigny C, Blier P. Increased tonic activation of rat forebrain 5-HT(1A) receptors by lithium addition to antidepressant treatments. Neuropsychopharmacology. 2000; 22(4): 346-356.
127. Haefely W, Burkard WP, Cesura A, Colzi A, Kettler R, Lorez HP, Martin JR, Moreau JL, Richards JG, Schaffner R, et al. Pharmacology of moclobemide. Clin Neuropharmacol. 1993; 16(Suppl 2): S8-S18.
128. Haefely W, Burkard WP, Cesura AM, Kettler R, Lorez HP, Martin JR, Richards JG, Scherschlicht R, Da Prada M.: Biochemistry and pharmacology of moclobemide, a prototype RIMA. Psychopharmacology (Berl). 1992; 106(Suppl): S6-S14.
129. Hagen T, Vidal-Puig A. Mitochondrial uncoupling proteins in human physiology and disease. Minerva Med. 2002; 93(1): 41-57.
130. Han YS, Lee CS. Antidepressants reveal differential effect against 1-methyl-4-phenylpyridinium toxocity in differentiated PC12 cells. Eur J Pharmacol. 2009; 604(1-3): 36-44.

131. Harper ME, Brand MD. Hyperthyroidism stimulates mitochondrial proton leak and ATP turnover in rat hepatocytes but does not change the overall kinetics of substrate oxidation reactions. Can J Physiol Pharmacol. 1994; 72(8): 899-908.
132. Harper ME, Brand MD. The quantitative contributions of mitochondrial proton leak and ATP turnover reactions to the changed respiration rates of hepatocytes from rats of different thyroid status. J Biol Chem 1993; 268(20): 14850-14860.
133. Heninger GR, Delgado PL, Charney DS. The revised monoamine theory of depression: a modulatory role for monoamines, based on new findings from monoamine depletion experiments in humans. Pharmacopsychiatry. 1996; 29(1): 2-11.
134. Hindmarch I. Beyond the monoamine hypothesis: mechanisms, molecules and methods. Eur Psychiatry. 2002; 17(Suppl 3): 294-299.
135. Hirst J. Towards the molecular mechanism of respiratory complex I. Biochem J. 2009; 425(2): 327-339.
136. Holford NH, Guentert TW, Dingemanse J, Banken L. Monoamine oxidase-A: pharmacodynamics in humans of moclobemide, a reversible and selective inhibitor. Br J Clin Pharmacol. 1994; 37(5): 433-439.
137. Honecker H, Christ W, Müller-Oerlinghausen B, Coper H. Critical evaluation of measurement of platelet monoamine oxidase in man. J Clin Chem Clin Biochem. 1976; 14(9): 453-458.
138. Horan MP, Pichaud N, Ballard JW. Review: Quantifying Mitochondrial Dysfunction in Complex Diseases of Aging. J Gerontol A Biol Sci Med Sci. 2012 [Epub ahead of print].
139. Horobin RW, Trapp S, Weissig V. Mitochondriotropics: a review of their mode of action, and their applications for drug and DNA delivery to mammalian mitochondria. J Control Release. 2007; 121(3): 125-136.
140. Horstink M, Tolosa E, Bonuccelli U, Deuschl G, Friedman A, Kanovsky P, Larsen JP, Lees A, Oertel W, Poewe W, Rascol O, Sampaio C, European Federation of Neurological Societies, Movement Disorder Society-European Section. Review of the therapeutic management of Parkinson's disease. Report of a joint task force of the European Federation of Neurological Societies and the Movement Disorder Society-European Section. Part I: early (uncomplicated) Parkinson's disease. Eur J Neurol. 2006; 13(11): 1170-1185.

141. Hovatta I, Juhila J, Donner J. Oxidative stress in anxiety and comorbid disorders. Neurosci Res. 2010; 68(4): 261-275.
142. Hroudova J, Fisar Z. Activities of respiratory chain complexes and citrate synthase influenced by pharmacologically different antidepressants and mood stabilizers. Neuro Endocrinol. Lett. 2010; 31(3): 336-342.
143. Hroudova J, Fisar Z. Connectivity between mitochondrial functions and psychiatric disorders. Psychiatry Clin Neurosci. 2011; 65(2): 130-141.
144. Huang RH, Faulkner R. The role of phospholipid in the multiple functional forms of brain monoamine oxidase. J Biol Chem. 1981; 256(17): 9211-9215.
145. Iwamoto K, Bundo M, Kato T. Altered expression of mitochondria-related genes in postmortem brains of patients with bipolar disorder or schizophrenia, as revealed by large-scale DNA microarray analysis. Hum Mol Genet. 2005; 14(2): 241-253.
146. Iwersen S, Schmoldt A. Three suicide attempts with moclobemide. J Toxicol Clin Toxicol. 1996; 34(2): 223-225.
147. Jacobs BL. Adult brain neurogenesis and depression. Brain Behav Immun. 2002; 16(5): 602-609
148. Jacobs BL, van Praag H, Gage FH. Adult brain neurogenesis and psychiatry: a novel theory of depression. Mol Psychiatry. 2000; 5(3): 262-269.
149. Jauch R, Griesser E, Oesterhelt G, Arnold W, Meister W, Ziegler WH, Guentert TW. Biotransformation of moclobemide in humans. Acta Psychiatr Scand Suppl. 1990; 360: 87-90.
150. Ježek P, Hlavatá L. Mitochondria in homeostasis of reactive oxygen species in cell, tissues, and organism. Int J Biochem Cell Biol. 2005; 37(12): 2478-2503.
151. Jiang X, Wang X. Cytochrome c promotes caspase-9 activation by inducing nucleotide binding to Apaf-1. J Biol Chem. 2000; 275(40): 31199-31203.
152. Jou SH, Chiu NY, Liu CS. Mitochondrial dysfunction and psychiatric disorders. Chang Gung Med J. 2009; 32(4): 370-379.
153. Joyce PR, Paykel ES. Predictors of drug response in depression. Arch Gen Psychiatry. 1989; 46(1): 89-99.

154. Kadenbach B, Hüttemann M, Arnold S, Lee I, Bender E. Mitochondrial energy metabolism is regulated via nuclear-coded subunits of cytochrome c oxidase. Free Radic Biol Med. 2000; 29(3-4): 211-221.
155. Kadenbach B, Ramzan R, Wen L, Vogt S. New extension of the Mitchell Theory for oxidative phosphorylation in mitochondria of living organisms.Biochim Biophys Acta. 2010; 1800(3): 205-212.
156. Kadenbach B. Intrinsic and extrinsic uncoupling of oxidative phosphorylation. Biochim Biophys Acta. 2003; 1604(2): 77-94.
157. Kamp F, Hamilton JA. pH gradients across phospholipid membranes caused by fast flip-flop of un-ionized fatty acids. Proc Natl Acad Sci U S A. 1992; 89(23): 11367-11370.
158. Karson CN, Newton JE, Livingston R, Jolly JB, Cooper TB, Sprigg J, Komoroski RA. Human brain fluoxetine concentrations. J Neuropsychiatry Clin Neurosci. 1993; 5(3): 322-329.
159. Kato T, Kakiuchi C, Iwamoto K. Comprehensive gene expression analysis in bipolar disorder. Can J Psychiatry. 2007; 52(12): 763-771.
160. Kato T, Kato N. Mitochondrial dysfunction in bipolar disorder. Bipolar Disord. 2000; 2(3 Pt 1): 180-190.
161. Kato T, Kunugi H, Nanko S, Kato N. Mitochondrial DNA polymorphisms in bipolar disorder. J Affect Disord. 2001; 62(3): 151-164.
162. Kato T. Mitochondrial dysfunction as the molecular basis of bipolar disorder: therapeutic implications. CNS Drugs. 2007; 21(1): 1-11.
163. Kato T. Role of mitochondrial DNA in calcium signaling abnormality in bipolar disorder. Cell Calcium. 2008; 44(1): 92-102.
164. Katyare SS, Rajan RR. Effect of long-term in vivo treatment with imipramine on the oxidative energy metabolism in rat brain mitochondria. Comp Biochem Psysiol C Pharmacol Toxicol Endocrinol. 1995; 112(3): 353-357.
165. Katyare SS, Rajan RR. Enhanced oxidative phosphorylation in rat liver mitochondria following prolonged in vivo treatment with imipramine. Br J Pharmacol. 1988; 95(3): 914-922.

166. Kempermann G, Kronenberg G. Depressed new neurons--adult hippocampal neurogenesis and a cellular plasticity hypothesis of major depression. Biol Psychiatry. 2003; 54(5): 499-503.
167. Kessler RC, Angermeyer M, Anthony JC, DE Graaf R, Demyttenaere K, Gasquet I, DE Girolamo G, Gluzman S, Gureje O, Haro JM, Kawakami N, Karam A, Levinson D, Medina Mora ME, Oakley Browne MA, Posada-Villa J, Stein DJ, Adley Tsang CH, Aguilar-Gaxiola S, Alonso J, Lee S, Heeringa S, Pennell BE, Berglund P, Gruber MJ, Petukhova M, Chatterji S, Ustün TB. Lifetime prevalence and age-of-onset distributions of mental disorders in the World Health Organization's World Mental Health Survey Initiative. World Psychiatry. 2007; 6(3): 168-176.
168. Kettler R, Da Prada M, Burkard WP. Comparison of monoamine oxidase-A inhibition by moclobemide in vitro and ex vivo in rats. Acta Psychiatr Scand Suppl. 1990; 360: 101-102.
169. Kim YJ, Ko HH, Han ES, Lee CS. Lamotrigine inhibition of rotenone- or 1-methyl-4-phenylpyridinium-induced mitochondrial damage and cell death. Brain Res Bull. 2007; 71(6): 633-640.
170. King TD, Bijur GN, Jope RS. Caspase-3 activation induced by inhibition of mitochondrial complex I is facilitated by glycogen synthase kinase-3beta and attenuated by lithium. Brain Res. 2001; 919(1): 106-114.
171. Kirby DM, Thorburn DR, Turnbull DM, Taylor RW. Biochemical assays of respiratory chain complex activity. In: Pon LA, Schon EA. (editors). Mitochondria, 2nd Edition (Methods in Cell Biology, Vol. 80). Elsevier Inc., San Diego, 2007. Pp. 93-119.
172. Kirkland RA, Franklin JL. Bax affects production of reactive oxygen by the mitochondria of non-apoptotic neurons. Exp Neurol. 2007; 204(1): 458-461.
173. Kispal G, Evans CT, Malloy C, Srere PA. Metabolic studies on citrate synthase mutants of yeast. A change in phenotype following transformation with an inactive enzyme. J Biol Chem. 1989; 264(19): 11204-11210.
174. Kitaichi Y, Inoue T, Nakagawa S, Izumi T, Koyama T. Effect of co-administration of subchronic lithium pretreatment and acute MAO inhibitors on extracellular monoamine levels and the expression of contextual conditioned fear in rats. Eur J Pharmacol. 2006; 532(3): 236-245.

175. Kleinfeld AM, Chu P, Romero C. Transport of long-chain native fatty acids across lipid bilayer membranes indicates that transbilayer flip-flop is rate limiting. Biochemistry. 1997; 36(46): 14146-14158.
176. Kolla N, Wei Z, Richardson JS, Li XM. Amitriptyline and fluoxetine protect PC12 cells from cell death induced by hydrogen peroxide. J Psychiatry Neurosci. 2005; 30(3): 196-201.
177. Konradi C, Eaton M, MacDonald ML, Walsh J, Benes FM, Heckers S. Molecular evidence for mitochondrial dysfunction in bipolar disorder. Arch Gen Psychiatry. 2004; 61(3): 300-308.
178. Konstantinov AA, Peskin AV, Popova EYu, Khomutov GB, Ruuge EK. Superoxide generation by the respiratory chain of tumor mitochondria. Biochim Biophys Acta. 1987; 894(1): 1-10.
179. Koopman WJ, Nijtmans LG, Dieteren CE, Roestenberg P, Valsecchi F, Smeitink JA, Willems PH. Mammalian mitochondrial complex I: biogenesis, regulation, and reactive oxygen species generation. Antioxid Redox Signal. 2010; 12(12): 1431-1470.
180. Krishnan V, Nestler EJ. The molecular neurobiology of depression. Nature. 2008; 455(7215): 894-902.
181. Kuznetsov AV, Schneeberger S, Seiler R, Brandacher G, Mark W, Steurer W, Saks V, Usson Y, Margreiter R, Gnaiger E. Mitochondrial defects and heterogeneous cytochrome c release after cardiac cold ischemia and reperfusion. Am J Physiol Heart Circ Physiol. 2004; 286(5): H1633-H1641.
182. Kwok KH, Ho PW, Chu AC, Ho JW, Liu HF, Yiu DC, Chan KH, Kung MH, Ramsden DB, Ho SL. Mitochondrial UCP5 is neuroprotective by preserving mitochondrial membrane potential, ATP levels, and reducing oxidative stress in MPP+ and dopamine toxicity. Free Radic Biol Med. 2010; 49(6): 1023-1035.
183. Lai JS, Zhao C, Warsh JJ, Li PP. Cytoprotection by lithium and valproate varies between cell types and cellular stresses. Eur J Pharmacol. 2006; 539(1-2): 18-26.
184. Lecrubier Y, Guelfi JD. Efficacy of reversible inhibitors of monoamine oxidase-A in various forms of depression. Acta Psychiatr Scand Suppl. 1990; 360: 18-23.
185. Lecrubier Y. Risk-benefit assessment of newer versus older monoamine oxidase (MAO) inhibitors. Drug Saf. 1994; 10(4): 292-300.

186. Lee I, Bender E, Arnold S, Kadenbach B. New control of mitochondrial membrane potential and ROS formation--a hypothesis. Biol Chem. 2001; 382(12): 1629-1636.
187. Lee I, Bender E, Kadenbach B. Control of mitochondrial membrane potential and ROS formation by reversible phosphorylation of cytochrome c oxidase. Mol Cell Biochem. 2002; 234-235(1-2): 63-70.
188. Lenaz G, Bovina C, D'Aurelio M, Fato R, Formiggini G, Genova ML, Giuliano G, Merlo Pich M, Paolucci U, Parenti Castelli G, Ventura B. Role of mitochondria in oxidative stress and aging. Ann N Y Acad Sci. 2002; 959: 199-213.
189. Leonard BE, Myint A. The psychoneuroimmunology of depression. Hum Psychopharmacol. 2009; 24(3): 165-175.
190. Levkovitz, Y, Gil-Ad I, Zeldich E, Dayag M, Weizman A. Differential induction of apoptosis by antidepressants in glioma and neuroblastoma cell lines: evidence for p-c-Jun, cytochrome c, and caspase-3 involvement. J Mol Neurosci. 2005; 27(1): 29-42.
191. Li Y, Couch L, Higuchi M, Fang JL, Guo L. Mitochondrial dysfunction induced by sertraline, an antidepressant agent. Toxicol Sci. 2012; 127(2): 582-591.
192. Lipton P. Ischemic cell death in brain neurons. Physiol Rev. 1999; 79(4): 1431-1568.
193. Liu D, Chan SL, de Souza-Pinto NC, Slevin JR, Wersto RP, Zhan M, Mustafa K, de Cabo R, Mattson MP. Mitochondrial UCP4 mediates an adaptive shift in energy metabolism and increases the resistance of neurons to metabolic and oxidative stress. Neuromolecular Med. 2006; 8(3): 389-414.
194. Lotufo-Neto F, Trivedi M, Thase ME. Meta-analysis of the reversible inhibitors of monoamine oxidase type A moclobemide and brofaromine for the treatment of depression. Neuropsychopharmacology. 1999; 20(3): 226-247.
195. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951; 193(1): 265-275.
196. Luder AS, Parks JK, Frerman F, Parker WD Jr. Inactivation of beef brain alpha-ketoglutarate dehydrogenase complex by valproic acid and valproic acid metabolites. J Clin Invest. 1990; 86(5): 1574-1581.
197. Ludwig B, Bender E, Arnold S, Hüttemann M, Lee I, Kadenbach B. Cytochrome C oxidase and the regulation of oxidative phosphorylation.Chembiochem. 2001; 2(6): 392-403.

198. Ma J, Qiu Y, Yang L, Peng L, Xia Z, Hou LN, Fang C, Qi H, Chen HZ. Desipramine induces apoptosis in rat glioma cells via endoplasmic reticulum stress-dependent CHOP pathway. J Neurooncol. 2011; 101(1): 41-48.
199. Maes M, Fišar Z, Medina M, Scapagnini G, Nowak G, Berk M. New drug targets in depression: inflammatory, cell-mediated immune, oxidative and nitrosative stress, mitochondrial, antioxidant, and neuroprogressive pathways. And new drug candidates-Nrf2 activators and GSK-3 inhibitors. Inflammopharmacology. 2012; 20(3): 127-150.
200. Maes M, Yirmyia R, Noraberg J, Brene S, Hibbeln J, Perini G, Kubera M, Bob P, Lerer B, Maj M. The inflammatory & neurodegenerative (I&ND) hypothesis of depression: leads for future research and new drug developments in depression. Metab Brain Dis. 2009; 24(1): 27-53.
201. Magder DM, Aleksic I, Kennedy SH. Tolerability and efficacy of high-dose moclobemide alone and in combination with lithium and trazodone. J Clin Psychopharmacol. 2000; 20(3): 394-395.
202. Magistretti PJ, Pellerin L. Cellular mechanisms of brain energy metabolism and their relevance to functional brain imaging. Philos Trans R Soc Lond B Biol Sci. 1999; 354(1387): 1155-1163.
203. Mai L, Jope RS, Li X. BDNF-mediated signal transduction is modulated by GSK3beta and mood stabilizing agents. J Neurochem. 2002; 82(1): 75-83.
204. Marazziti D, Baroni S, Picchetti M, Landi P, Silvestri S, Vatteroni E, Catena Dell'Osso M. Psychiatric disorders and mitochondrial dysfunctions. Eur Rev Med Pharmacol Sci. 2012; 16(2): 270-275.
205. Markham A, Cameron I, Franklin P, Spedding M. BDNF increases rat brain mitochondrial respiratory coupling at complex I, but not complex II. Eur. J Neurosci. 2004; 20(5): 1189-1196.
206. Mathew SJ, Manji HK, Charney DS. Novel drugs and therapeutic targets for severe mood disorders. Neuropsychopharmacology. 2008; 33(9): 2080-2092.
207. Mattson MP, Gleichmann M, Cheng A. Mitochondria in neuroplasticity and neurological disorders. Neuron. 2008; 60(5): 748-766.
208. Mattson MP, Partin J. Evidence for mitochondrial control of neuronal polarity. J Neurosci Res. 1999; 56(1): 8-20.

209. Maurer IC, Schippel P, Volz HP. Lithium-induced enhancement of mitochondrial oxidative phosphorylation in human brain tissue. Bipolar Disord. 2009; 11(5): 515-522.
210. McCormack JG, Halestrap AP, Denton RM. Role of calcium ions in regulation of mammalian intramitochondrial metabolism. Physiol Rev. 1990; 70(2): 391-425.
211. Mendelsohn D, Riedel WJ, Sambeth A. Effects of acute tryptophan depletion on memory, attention and executive functions: a systematic review. Neurosci Biobehav Rev. 2009; 33(6): 926-952.
212. Merlo-Pich M, Deleonardi G, Biondi A, Lenaz G. Methods to detect mitochondrial function. Exp Gerontol. 2004; 39(3): 277-281.
213. Mitchell P. Chemiosmotic coupling in oxidative and photosynthetic phosphorylation, Biol Rev Camb Philos Soc. 1966; 41(3): 445-502.
214. Mitchell P. Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. Nature. 1961; 191: 144-148.
215. Moghaddam B. Stress activation of glutamate neurotransmission in the prefrontal cortex: implications for dopamine-associated psychiatric disorders. Biol Psychiatry. 2002; 51(10): 775-787.
216. Morota S, Månsson R, Hansson MJ, Kasuya K, Shimazu M, Hasegawa E, Yanagi S, Omi A, Uchino H, Elmér E. Evaluation of putative inhibitors of mitochondrial permeability transition for brain disorders--specificity vs. toxicity. Exp Neurol. 2009; 218(2): 353-362.
217. Mourier A, Devin A, Rigoulet M. Active proton leak in mitochondria: a new way to regulate substrate oxidation. Biochim Biophys Acta. 2010; 1797:255-261.
218. Müller U, Troidl C, Niemann S. SDHC mutations in hederitary paraganglioma/pheochromocytoma. Fam Cancer. 2005; 4(1): 9-12.
219. Mukherjee J, Yang ZY. Evaluation of monoamine oxidase B inhibition by fluoxetine (Prozac): an in vitro and in vivo study. Eur J Pharmacol. 1997; 337(1): 111-114.
220. Mukherjee J, Yang Z-Y.: Monoamine oxidase A inhibition by fluoxetine: an in vitro and in vivo study. Synapse. 1999; 31(4): 285-289.
221. Muller FL, Liu Y, Van Remmen H. Complex III releases superoxide to both sides of the inner mitochondrial membrane. J Biol Chem. 2004; 279(47): 49064-49073.

222. Munujos P, Coll-Cantí J, González-Sastre F, Gella FJ. Assay of succinate dehydrogenase activity by a colorimetric-continuous method using iodonitrotetrazolium chloride as electron acceptor. Anal Biochem. 1993; 212(2): 506-509.
223. Muraki I, Inoue T, Hashimoto S, Izumi T, Ito K, Koyama T. Effect of subchronic lithium treatment on citalopram-induced increases in extracellular concentrations of serotonin in the medial prefrontal cortex. J Neurochem. 2001; 76(2): 490-497.
224. Muriel P, Pérez-Rojas JM. Nitric oxide inhibits mitochondrial monoamine oxidase activity and decreases outer mitochondria membrane fluidity. Comp Biochem Physiol C Toxicol Pharmacol. 2003; 136(3): 191-197.
225. Murphy MP. How mitochondria produce reactive oxygen species. Biochem J. 2009; 417(1): 1-13.
226. Nag M. Effect of chlorpromazine, imipramine and lithium on MAO-A and MAO-B activity in rat brain mitochondria. Indian J Exp Biol. 2004; 42(9): 941-944.
227. Nair NP, Ahmed SK, Kin NM. Biochemistry and pharmacology of reversible inhibitors of MAO-A agents: focus on moclobemide. J Psychiatr Neurosci. 1993; 18(5): 214-225.
228. Naoi M, Maruyama W, Akao Y, Yi H, Yamaoka Y. Involvement of type A monoamine oxidase in neurodegeneration: regulation of mitochondrial signaling leading to cell death or neuroprotection. J Neural Transm Suppl. 2006; (71): 67-77.
229. Napiwotzki J, Kadenbach B: Extramitochondrial ATP/ADP-ratios regulate cytochrome c oxidase activity via binding to the cytosolic domain of subunit IV. Biol Chem. 1998; 379(3): 335-339.
230. Nestler EJ, Barrot M, DiLeone RJ, Eisch AJ, Gold SJ, Monteggia LM. Neurobiology of depression. Neuron. 2002; 34(1): 13-25.
231. Nicholls DG, Vesce S, Kirk L, Chalmers S. Interactions between mitochondrial bioenergetics and cytoplasmic calcium in cultured cerebellar granule cells. Cell Calcium. 2003; 34(4-5): 407-424.
232. Nierenberg AA, Fava M, Trivedi MH, Wisniewski SR, Thase ME, McGrath PJ, Alpert JE, Warden D, Luther JF, Niederehe G, Lebowitz B, Shores-Wilson K, Rush AJ. A comparison of lithium and T_3 augmentation following two failed medication treatments for depression: a STAR*D report. Am J Psychiatry. 2006; 163(9): 1519-1530.

233. Noji H, Yoshida M. The rotary machine in the cell, ATP synthase.J Biol Chem. 2001; 276(3): 1665-1668.
234. Nůsková H, Vrbacký M, Drahota Z, Houštěk J. Cyanide inhibition and pyruvate-induced recovery of cytochrome c oxidase. J Bioenerg Biomembr. 2010; 42(5): 395-403.
235. Orth M, Schapira AH. Mitochondria and degenerative disorders. Am J Med Genet. 2001; 106(1): 27-36.
236. Overly CC, Rieff HI, Hollenberck PJ. Organelle motility and metabolism in axons vs dendrites of cultured hippocampal neurons. J Cell Sci. 1996; 109(Pt 5): 971-980.
237. Ozaita A, Olmos G, Boronat MA, Lizcano JM, Unzeta M, García-Sevilla JA. Inhibition of monoamine oxidase A and B activities by imidazol(ine)/guanidine drugs, nature of the interaction and distinction from I_2-imidazoline receptors in rat liver. Br J Pharmacol. 1997; 121(5): 901-912.
238. Papa S, Scacco S, Sardanelli AM, Petruzzella V, Vergari R, Signorile A, Technikova-Dobrovova Z. Complex I and the cAMP cascade in human physiopathology. Biosci Rep. 2002; 22(1): 3-16.
239. Pathak RU, Davey GP. Complex I and energy thresholds in the brain. Biochim Biophys Acta. 2008; 1777(7-8): 777-782.
240. Pesta D, Gnaiger E. High-resolution respirometry: OXPHOS protocols for human cells and permeabilized fibers from small biopsies of human muscle. Methods Mol Biol. 2012; 810: 25-58.
241. Petrosillo G, Matera M, Casanova G, Ruggiero FM, Paradies G. Mitochondrial dysfunction in rat brain with aging Involvement of complex I, reactive oxygen species and cardiolipin. Neurochem Int. 2008; 53(5): 126-131.
242. Pittenger C, Duman RS. Stress, depression, and neuroplasticity: a convergence of mechanisms. Neuropsychopharmacology. 2008; 33(1): 88-109.
243. Ponchaut S, van Hoof F, Veitch K. In vitro effects of valproate and valproate metabolites on mitochondrial oxidations. Relevance of CoA sequestration to the observed inhibitions. Biochem Pharmacol. 1992; 43(11): 2435-2442.
244. Praško J, Dockery C, Horáček J, Houbová P, Kosová J, Klaschka J, Pašková B, Prašková H, Seifertová D, Záleský R, Höschl C. Moclobemide and cognitive behavioral therapy in

the treatment of social phobia. A six-month controlled study and 24 months follow up. Neuro Endocrinol Lett. 2006; 27(4): 473-481.

245. Quiroz JA, Gray NA, Kato T, Manji HK. Mitochondrially mediated plasticity in the pathophysiology and treatment of bipolar disorder. Neuropsychopharmacology. 2008; 33(11): 2551-2565.
246. Ragan CI, Wilson MT, Darley-Usmar VM, Lowe PN. Subfractionation of mitochondria and isolation of the proteins of oxidative phosphorylation. In: Darley-Usmar VM, Rickwood D, Wilson MT, editors. Mitochondria. A Practical Approach. IRL Press, London, 1987. Pp. 79-112.
247. Raha S, McEachern GE, Myint AT, Robinson BH. Superoxides from mitochondrial complex III: the role of manganese superoxide dismutase. Free Radic Biol Med. 2000; 29(2): 170-180.
248. Rajkowska G. Cell pathology in bipolar disorder. Bipolar Disord. 2002; 4(2): 105-116.
249. Ramsay RR, Gravestock MB. Monoamine oxidases: to inhibit or not to inhibit. Mini Rev Med Chem. 2003; 3(2): 129-136.
250. Ramzan R, Staniek K, Kadenbach B, Vogt S. Mitochondrial respiration and membrane potential are regulated by the allosteric ATP-inhibition of cytochrome c oxidase. Biochim Biophys Acta 2010; 1797(9): 1672-1680.
251. Redrobe JP, Bourin M. Evidence of the activity of lithium on 5-HT1B receptors in the mouse forced swimming test: comparison with carbamazepine and sodium valproate. Psychopharmacology (Berl). 1999; 141(4): 370-377.
252. Reid AA, Hill JL, Murphy DL. Interactions of tricyclic antidepressant drugs with human and rat monoamine oxidase type B. Naunyn Schmiedebergs Arch Pharmacol. 1988; 338(6): 678-683.
253. Reinecke F, Smeitink JA, van der Westhuizen FH. OXPHOS gene expression and control in mitochondrial disorders. Biochim Biophys Acta. 2009; 1792(12): 1113-1121.
254. Reveley MA, Glover V, Sandler M, Coppen A. Increased platelet monoamine oxidase activity in affective disorders. Psychopharmacology (Berl). 1981; 73(3): 257-260.
255. Reveley MA, Glover V, Slander M, Coppen A. Absence of platelet monamine oxidase inhibition during amitriptyline or zimelidine treatment. Br J Clin Pharmacol. 1979; 8(4): 375-378.

256. Reyes E, Lisansky J. Effects of tricyclic antidepressants on platelet monoamine oxidase activity. Clin Pharmacol Ther. 1984; 35(4): 531-534.
257. Rezin GT, Amboni G, Zugno AI, Quevedo J, Streck EL. Mitochondrial dysfunction and psychiatric disorders. Neurochem Res. 2009; 34(6): 1021-1029.
258. Rial E, Rodríguez-Sánchez L, Gallardo-Vara E, Zaragoza P, Moyano E, González-Barroso MM. Lipotoxicity, fatty acid uncoupling and mitochondrial carrier function. Biochim Biophys Acta. 2010; 1797(6-7): 800-806.
259. Riederer P, Danielczyk W, Grünblatt E. Monoamine oxidase-B inhibition in Alzheimer's disease. NeuroToxicology. 2004; 25(1-2): 271-277.
260. Rizzuto R, Bernardi P, Pozzan T. Mitochondria as all-round players of the calcium game. J Physiol 2000; 529 Pt 1:37-47.
261. Rodríguez-Hernández A, Cordero MD, Salviati L, Artuch R, Pineda M, Briones P, Gómez Izquierdo L, Cotán D, Navas P, Sánchez-Alcázar JA. Coenzyme Q deficiency triggers mitochondria degradation by mitophagy. Autophagy. 2009; 5(1): 19-32.
262. Ross MF, Da Ros T, Blaikie FH, Prime TA, Porteous CM, Severina II, Skulachev VP, Kjaergaard HG, Smith RA, Murphy MP. Accumulation of lipophilic dications by mitochondria and cells. Biochem J. 2006; 400(1): 199-208.
263. Roth JA, Gillis CN. Inhibition of lung, liver and brain monoamine oxidase by imipramine and desipramine. Biochem Pharmacol. 1974; 23(6): 1138-1140.
264. Ruhé HG, Mason NS, Schene AH. Mood is indirectly related to serotonin, norepinephrine and dopamine levels in humans: a meta-analysis of monoamine depletion studies. Mol Psychiatry. 2007; 12(4): 331-359.
265. Rustin P, Chretien D, Bourgeron T, Gérard B, Rötig A, Saudubray JM, Munnich A. Biochemical and molecular investigations in respiratory chain deficiencies. Clin Chim Acta. 1994; 228(1): 35-51.
266. Ruthel G, Hollenberck PJ. Response of mitochondrial traffic to axon determination and differential branch growth. J Neurosci. 2003; 23(24): 8618-8624.
267. Saada A, Shaag A, Mandel H, Nevo Y, Eriksson S, Elpeleg O. Mutant mitochondrial thymidine kinase in mitochondrial DNA depletion myopathy. Nat Genet. 2001; 29(3): 342-344.

268. Samartsev VN, Marchik EI, Shamagulova LV. Free fatty acids as inducers and regulators of uncoupling of oxidative phosphorylation in liver mitochondria with participation of ADP/ATP- and aspartate/glutamate-antiporter. Biochemistry (Mosc). 2011; 76(2): 217-224.
269. Santos NA, Medina WS, Martins NM, Mingatto FE, Curti C, Santos AC. Aromatic antiepileptic drugs and mitochondrial toxicity: effects on mitochondria isolated from rat liver. Toxicol In Vitro. 2008; 22(5): 1143-52.
270. Scaini G, Santos PM, Benedet J, Rochi N, Gomes LM, Borges LS, Rezin GT, Pezente DP, Quevedo J, Streck EL. Evaluation of Krebs cycle enzymes in the brain of rats after chronic administration of antidepressants. Brain Res Bull. 2010; 82(3-4): 224-227.
271. Schapira AHV. Mitochondrial diseases. Lancet. 2012; 379(9828): 1825-1834.
272. Schapira AHV. Mitochondrial dysfunction in neurodegenerative disorders. Biochim Biophys Acta. 1998; 1366(1-2): 225-233.
273. Scheffler IE. Molecular genetics of succinate: quinone oxidoreductase in eukaryotes. Prog Nucleic Acid Res Mol Biol. 1998; 60: 267-315.
274. Schildkraut JJ. The catecholamine hypothesis of affective disorders: a review of supporting evidence. Am J Psychiatry. 1965; 122(5): 509-522.
275. Schönfeld P, Wojtczak L. Fatty acids as modulators of the cellular production of reactive oxygen species. Free Radic Biol Med. 2008; 45(3): 231-241.
276. Shalbuyeva N, Brustovetsky T, Brustovetsky N. Lithium desensitizes brain mitochondria to calcium, antagonizes permeability transition, and diminishes cytochrome C release. J Biol Chem. 2007; 282(25): 18057-18068.
277. Shaltiel G, Chen G, Manji HK. Neurotrophic signaling cascades in the pathophysiology and treatment of bipolar disorder. Curr Opin Pharmacol. 2007; 7(1): 22-26.
278. Shao L, Martin MV, Watson SJ, Schatzberg A, Akil H, Myers RM, Jones EG, Bunney WE, Vawter MP. Mitochondrial involvement in psychiatric disorders. Ann Med. 2008; 40(4): 281-295.
279. Silva MF, Ruiter JP, Illst L, Jakobs C, Duran M, de Almeida IT, Wanders RJ. Valproate inhibits the mitochondrial pyruvate-driven oxidative phosphorylation in vitro. J Inherit Metab Dis. 1997; 20(3): 397-400.

280. Sjövall F, Morota S, Hansson MJ, Friberg H, Gnaiger E, Elmér E. Temporal increase of platelet mitochondrial respiration is negatively associated with clinical outcome in patients with sepsis. Crit Care. 2010; 14(6): R214.
281. Smith TE, Weisssbach H, Udenfriend S. Studies on monoamine oxidase: the mechanism of inhibition of monoamine oxidase by iproniazid. Biochemistry. 1963; 2(4): 746-751.
282. Solmaz SR, Hunte C. Structure of complex III with bound cytochrome c in reduced state and definition of a minimal core interface for electron transfer. J Biol Chem. 2008; 283(25): 17542-17549.
283. Souza ME, Polizello AC, Uyemura SA, Castro-Silva O, Curti C. Effect of fluoxetine on rat liver mitochondria. Biochem Pharmacol. 1994; 48(3): 535-541.
284. Srere PA. Citrate synthase: [EC 4.1.3.7. Citrate oxaloacetate-lyase (CoA-acetylating)]. Methods Enzymol. 1969; 13: 3-11.
285. Sriram G, Martinez JA, McCabe ER, Liao JC, Dipple KM. Single-gene disorders: what role could moonlighting enzymes play? Am J Hum Genet. 2005; 76(6): 911-924.
286. Stahl SM, Felker A. Monoamine oxidase inhibitors: a modern guide to an unrequited class of antidepressants. CNS Spectr. 2008; 13(10): 855-870.
287. Stamer K, Vogel R, Thies E, Mandelkow E, Mandelkow EM. Tau blocks traffic of organelles, neurofilaments, and APP vesicles in neurons and enhances oxidative stress. J Cell Biol. 2002; 156(6): 1051-1063.
288. Starkov AA. "Mild" uncoupling of mitochondria. Biosci Rep. 1997; 17(3): 273-279.
289. Starkov AA. The role of mitochondria in reactive oxygen species metabolism and signaling. Ann N Y Acad Sci. 2008; 1147: 37-52.
290. Stork C, Renshaw RF. Mitochondrial dysfunction in bipolar disorder: evidence from magnetic resonance spectroscopy research. Mol Psychiatry. 2005; 10(10): 900-919.
291. Strakowski SM, Delbello MP, Adler CM. The functional neuroanatomy of bipolar disorder: a review of neuroimaging findings. Mol Psychiatry. 2005; 10(1): 105-116.
292. Sullivan JL, Dackis C, Stanfield C. In vivo inhibition of platelet MAO activity by tricyclic antidepressants. Am J Psychiatry. 1977; 134(2): 188-190.
293. Sullivan JL, Zung WW, Stanfield CN, Cavenar JO Jr. Clinical correlates of tricyclic antidepressant-mediated inhibition of platelet monoamine oxidase. Biol Psychiatry. 1978; 13(3): 399-407.

294. Szewczyk A, Wojtczak L. Mitochondria as a pharmacological target. Pharmacol Rev. 2002; 54(1): 101-127.
295. Tager JM, Wanders RJ, Groen AK, Kunz W, Bohnensack R, Küster U, Letko G, Böhme G, Duszynski J, Wojtczak L. Control of mitochondrial respiration. FEBS Lett. 1983; 151(1): 1-9.
296. Telford JE, Kilbride SM, Davey GP. Complex I is rate-limiting for oxygen consumption in the nerve terminal. J Biol Chem. 2009; 284(14): 9109-9114.
297. Tirosh O, Aronis A, Melendez JA. Mitochondrial state 3 to 4 respiration transition during Fas-mediated apoptosis controls cellular redox balance and rate of cell death. Biochem Pharmacol. 2003; 66(8): 1331-1334.
298. Tomitsuka E, Kita K, Esumi H. Regulation of succinate-ubiquinone reductase and fumarate reductase activities in human complex II by phosphorylation of its flavoprotein subunit.Proc Jpn Acad Ser B Phys Biol Sci. 2009; 85(7): 258-265.
299. Trenker M, Malli R, Fertschai I, Levak-Frank S, Graier WF. Uncoupling proteins 2 and 3 are fundamental for mitochondrial Ca2+ uniport. Nat Cell Biol. 2007; 9(4): 445-452.
300. Tretter L, Adam-Vizi V. Generation of reactive oxygen species in the reaction catalyzed by alpha-ketoglutarate dehydrogenase. J Neurosci. 2004; 24(36): 7771-7778.
301. Trounce IA, Kim YL, Jun AS, Wallace DC. Assessment of mitochondrial oxidative phosphorylation in patient muscle biopsies, lymphoblasts, and transmitochondrial cell lines. Methods Enzymol. 1996; 264: 484-509.
302. Trumpower BL. The protonmotive Q cycle. Energy transduction by coupling of proton translocation to electron transfer by the cytochrome bc_1 complex. J Biol Chem. 1990; 265(20): 11409-11412.
303. Tsujimoto Y, Shimizu S. Role of the mitochondrial membrane permeability transition in cell death. Apoptosis. 2007; 12(5): 835-840.
304. Turrens JF. Mitochondrial formation of reactive oxygen species. J Physiol. 2003; 552(Pt 2): 335-344.
305. Ulus IH, Maher TJ, Wurtman RJ. Characterization of phentermine and related compounds as monoamine oxidase (MAO) inhibitors. Biochem Pharmacol. 2000; 59(12): 1611-1621.
306. Valvassori SS, Rezin GT, Ferreira CL, Moretti M, Gonçalves CL, Cardoso MR, Streck EL, Kapczinski F, Quevedo J. Effects of mood stabilizers on mitochondrial respiratory

chain activity in brain of rats treated with d-amphetamine. J Psychiatr Res. 2010; 44(14): 903-909.

307. Vawter MP, Tomita H, Meng F, Bolstad B, Li J, Evans S, Choudary P, Atz M, Shao L, Neal C, Walsh DM, Burmeister M, Speed T, Myers R, Jones EG, Watson SJ, Akil H, Bunney WE. Mitochondrial-related gene expression changes are sensitive to agonal-pH state: implications for brain disorders. Mol Psychiatry. 2006; 11(7): 663-679.
308. Velasco A, González-Calvo V, Alvarez FJ, Dueñas A, García-Roldán JL. Effect of trazodone on oxidative metabolism of rat brain in vitro. Rev Esp Fisiol. 1985; 41(2): 201-205.
309. Vélot C, Srere PA. Reversible transdominant inhibition of a metabolic pathway. In vivo evidence of interaction between two sequential tricarboxylic acid cycle enzymes in yeast. J Biol Chem. 2000; 275(17): 12926-12933.
310. Videbech P. PET measurements of brain glucose metabolism and blood flow in major depressive disorder: a critical review. Acta Psychiatr Scand. 2000; 101(1): 11-20.
311. Viola HM, Hool LC. Qo site of mitochondrial complex III is the source of increased superoxide after transient exposure to hydrogen peroxide. J Mol Cell Cardiol. 2010; 49(5): 875-885.
312. Vonck J, Schäfer E. Supramolecular organization of protein complexes in the mitochondrial inner membrane. Biochim Biophys Acta. 2009; 1793(1): 117-124.
313. Walsh C, Barrow S, Voronina S, Chvanov M, Petersen OH, Tepikin A. Modulation of calcium signalling by mitochondria. Biochim Biophys Acta. 2009; 1787(11): 1374-1382.
314. Wang H, Guan Y, Wang X, Smith K Cormier K, Zhu S, Stavrovskaya IG, Huo C, Ferrante RJ, Kristal BS, Friedlander RM. Nortriptyline delays disease onset in models of chronic neurodegeneration. Eur J Neurosci. 2007; 26(3): 633-641.
315. Wang JF. Defects of mitochondrial electron transport chain in bipolar disorder: implications for mood-stabilizing treatment. Can J Psychiatry. 2007; 52(12): 753-762.
316. Weinbach EC, Costa JL, Nelson BD, Claggett CE, Hundal T, Bradley D, Morris SJ. Effects of tricyclic antidepressant drugs on energy-linked reactions in mitochondria. Biochem Pharmacol. 1986; 35(9): 1445-1451.
317. Whittaker VP. The synaptosome. In: Handbook of Neurochemistry (Vol. II). Lajtha A (editor). Plenum Press, New York, 1969. Pp. 327-364.

318. Wilson DF, Chance B. Azide inhibition of mitochondrial electron transport. I. The aerobic steady state of succinate oxidation. Biochim Biophys Acta. 1967; 131(3): 421-430.
319. Wojtczak L, Schönfeld P. Effect of fatty acids on energy coupling processes in mitochondria. Biochim Biophys Acta. 1993; 1183(1): 41-57.
320. Wolkow CA, Iser WB. Uncoupling protein homologs may provide a link between mitochondria, metabolism and lifespan. Ageing Res Rev. 2006; 5(2): 196-208.
321. Wong-Riley MT. Cytochrome oxidase: an endogenous metabolic marker for neuronal activity. Trends Neurosci. 1989; 12(3): 94-101.
322. Xia Z, Karlsson H, DePierre JW, Nässberger L. Tricyclic antidepressants induce apoptosis in human T lymphocytes. Int J Immunopharmacol. 1997; 19(11-12): 645-654.
323. Xia Z, Lundgren B, Bergstrand A, DePierre JW, Nässberger L. Changes in the generation of reactive oxygen species and in mitochondrial membrane potential during apoptosis induced by the antidepressants imipramine, clomipramine, and citalopram and the effects on these changes by Bcl-2 and Bcl-X(L). Biochem Pharmacol. 1999; 57(10): 1199-1208.
324. Youdim MB, Bakhle YS. Monoamine oxidase: isoforms and inhibitors in Parkinson's disease and depressive illness. Br J Pharmacol. 2006; 147(Suppl 1): S287-S296.
325. Youdim MB, Edmondson D, Tipton KF. The therapeutic potential of monoamine oxidase inhibitors. Nat Rev Neurosci. 2006; 7(4): 295-309.
326. Zaninovich AA. Role of uncoupling proteins UCP1, UCP2 and UCP3 in energy balance, type 2 diabetes and obesity. Synergism with the thyroid]. Medicina (B Aires). 2005; 65(2): 163-169.
327. Zanotti F, Gnoni A, Mangiullo R, Papa S. Effect of the ATPase inhibitor protein IF_1 on H^+ translocation in the mitochondrial ATP synthase complex. Biochem Biophys Res Commun. 2009; 384(1): 43-48.
328. Zarate CA Jr, Singh J, Manji HK. Cellular plasticity cascades: targets for the development of novel therapeutics for bipolar disorder. Biol Psychiatry. 2006; 59(11): 1006-1020.
329. Zhang WH, Wang H, Wang X, Narayanan MV, Stavrovskaya IG, Kristal BS, Friedlander RM. Nortriptyline protects mitochondria and reduces cerebral ischemia/hypoxia injury. Stroke. 2008; 39(2): 455-462.

330. Zugno AI, Valvassori SS, Scherer EB, Mattos C, Matté C, Ferreira CL, Rezin GT, Wyse AT, Quevedo J, Streck EL. Na+,K+-ATPase activity in an animal model of mania. J Neural Transm. 2009; 116(4): 431-436.

List of publications

1. Publications *in extenso* related to this thesis

a) with IF

Hroudová J, Fišar 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 Biologica (Praha). 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**, Raboch J. Mitochondrie a poruchy nálady. In Raboch J., Zrzavecká I., Doubek P. (pořadatelé). Duševní poruchy a kvalita péče. Sborník přednášek a abstrakt VIII. sjezdu Psychiatrické společnosti ČLS JEP s mezinárodní účastí. 1. vydání, Brno: Tribun EU, 2010; ISBN 978-80-7399-958-2, p. 79-82.

Hroudová J, Fišar Z, Raboch J, Hansíková H. Vliv antidepresiv na aktivitu mitochondriálních enzymů. In Raboch J, Zrzavecká I, Doubek P (pořadatelé). Duševní poruchy a kvalita péče. Sborník přednášek a abstrakt VIII. sjezdu Psychiatrické společnosti ČLS JEP s mezinárodní účastí. 1. vydání, Brno: Tribun EU, 2010; ISBN 978-80-7399-958-2, p. 106-109.

Hroudová J, Fišar Z, Raboch J, Hansíková H. Změny aktivit monoaminoxidasy, citrátsyntasy a enzymů respiračního řetězce způsobené antidepresivy a stabilizátory nálady. In Raboch J, Zrzavecká I, Doubek P (pořadatelé). Duševní poruchy a kvalita péče. Sborník přednášek a abstrakt VIII. sjezdu Psychiatrické společnosti ČLS JEP s mezinárodní účastí. 1. vydání, Brno: Tribun EU, 2010; ISBN 978-80-7399-958-2, p. 110-112.

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.

Hroudová J. Vliv antidepresiv a stabilizátorů nálady na mitochondriální funkce abstrakt přednášky In sborník 12. studentská vědecká konference; Oddělení vnějších vztahů 1. LF UK. Praha: Nakladatelství Galén, s.r.o., 2011; ISBN 978-80-7262-822-3, p. 34–34.

Fišar Z, Wenchich L, Pláteník J, Buchal R, Kitzlerová E, Zvěřová M, Jirák R, **Hroudová J**, Raboch J. Monoaminergní systém, neurotrofní faktory a buněčná energetika při depresivní poruše. In: Společně na cestě k moderní psychiatrii. Sborník příspěvků 15. česko-slovenského psychiatrického sjezdu s mezinárodní účastí. Brno, Tribun EU, 2011; ISBN 978-80-263-0039-7, p. 185-188.

Fišar Z, Jirák R, Pláteník J, Wenchich L, Buchal R, Kitzlerová E, Zvěřová M, **Hroudová J**, Raboch J. Alzheimerova porucha, depresivní porucha a změny v nitrobuněčných signálních cestách. In: Sborník příspěvků 15. česko-slovenského psychiatrického sjezdu s mezinárodní účastí. Brno, Tribun EU, 2011; ISBN 978-80-263-0039-7, p. 235-237.

Jirák R, Fišar Z, Wenchich L, Pláteník J, Buchal R, Zvěřová M, **Hroudová J**, Raboch J. Změny nitrobuněčných signálních cest při Alzheimerově poruše. In: Sborník příspěvků 15. česko-slovenského psychiatrického sjezdu s mezinárodní účastí. Brno, Tribun EU, 2011; ISBN 978-80-263-0039-7, p. 39-41.

Hroudová J, Fišar Z, Raboch J. Změny energetického metabolismu buňky vlivem psychofarmak a psychiatrických onemocnění In Sborník příspěvků IX. sjezdu Psychiatrické společnosti ČLS JEP s mezinárodní účastí. 1. vydání, Brno: Tribun EU, 2012; ISBN 978-80-263-0243-8, p. 267-269.

Hroudová J, Fišar Z, Jirák R, Wenchich L, Raboch J. Změny buněčného metabolismi u pacientů s Alzheimerovou chorobou. In Sborník příspěvků IX. sjezdu Psychiatrické společnosti ČLS JEP s mezinárodní účastí. 1. vydání, Brno: Tribun EU, 2012; ISBN 978-80-263-0243-8, p. 49-51.

2. Publications *in extenso* unrelated to this thesis

a) with IF

Korabecny 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 MF, Salinin S, Ridder DA, 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 YS, 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. Bioorganic & Medicinal Chemistry Letters. 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