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Mitochondrial functions in neuropsychopharmacology

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Content

Abbreviations

7-MEOTA - 7-methoxytacrine
ABAD - amyloid-beta binding alcohol dehydrogenase
Aβ - β-amyloid
AChE - acetylcholinesterase
AD - Alzheimer's disease
APP - amyloid precursor protein
ATP - adenosine 5´-triphosphate
BChE - butyrylcholinesterase
Bcl-2 - B-cell CLL/lymphoma 2
BD - bipolar disorder
BDNF - brain-derived neurotrophic factor
BPRS - brief psychiatric rating scale
cAMP - 3´,5´-cyclic adenosine monophosphate
CGI-I – clinical global impression – Improvement scale
CGI-S - clinical global impression – Severity scale
ChE - cholinesterase
CNS - central nervous system
COMT - catechol-*O*-methyltransferase
CoQ_{10} - coenzyme Q_{10}, ubiquinone
COX - cytochrome *c* oxidase
CREB - cAMP response element binding protein
CS - citrate synthase
CypD - cyclophilin D
cyt *c* - cytochrome *c*
ETC - electron transport chain
ETS - electron transfer system
ETSC - maximal capacity of the electron transfer system
FAD - flavin adenine dinucleotide
FCCP - *p*-(trifluoromethoxy)phenylhydrazone
GSK - glycogen synthase kinase
H_2O_2 - hydrogen peroxide
HPA - hypothalamic-pituitary-adrenal
HRSD - Hamilton rating scale for depression
IC_{50} - half maximal inhibitory concentration
K_i - dissociation constant of an enzyme-inhibitor complex
K_m - Michaelis constant
LEAK - state 4, non-phosphorylating resting state of intrinsic uncoupled respiration
M_2 - muscarinic acetylcholine receptor, type 2
MAO - monoamine oxidase
MAO-A, MAO-B - isoforms of monoamine oxidase

MASSA - melatonin agonist and selective serotonin antagonist
MDD - major depressive disorder
Mfn1, Mfn2 - mitofusin 1, mitofusin 2
MMSE - Mini Mental State Examination
MPP^+ - 1-methyl-4-phenylpyridinium
MPT - mitochondrial permeability transition
mPTP - mitochondrial permeability transition pores
MRS - magnetic resonance spectroscopy
mtDNA - mitochondrial DNA
NADH - reduced nicotinamide adenine dinucleotide
NaSSA - noradrenergic and specific serotonergic antidepressant
nDNA - nuclear DNA
NDRI - norepinephrine-dopamine reuptake inhibitor
NE - norepinephrine, syn. noradrenaline
NetPR - net physiological respiration
NGF - nerve growth factor
NMDA - *N*-methyl-D-aspartic acid
NRI - norepinephrine reuptake inhibitor
OPA1 - optic atrophy factor 1
OXPHOS - oxidative phosphorylation
PD - Parkinson's disease
PEPSI - proton echo-planar spectroscopic imaging
PET - positron emission tomography
PR - physiological respiration
ROS - reactive oxygen species
Rot - respiration after complex I inhibition, induced by rotenone
ROX - residual oxygen consumption
SARI - serotonin antagonist and reuptake inhibitor
SDH - succinate dehydrogenase
SERT - serotonin transporter
SH-SY5Y - human neuroblastoma cells
SNRI - serotonin-norepinephrine reuptake inhibitor
SPECT - single-photon emission computed tomography
SSRE - selective serotonin reuptake enhancer
SSRI - selective serotonin reuptake inhibitor
SZ - schizophrenia
TCA - tricarboxylic acid
V_{max} - maximum rate of enzymatic reaction
YMRS - Young Mania Rating Scale
$\Delta\psi_m$ - mitochondrial membrane potential

Introduction

1. Mitochondrial dysfunctions and psychiatric disorders

It is well-known that mitochondria strongly affect many intracellular processes related 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, such as major depressive disorder (MDD), bipolar disorder (BD), schizophrenia (SZ), psychosis, anxiety disorder and borderline personality disorders [1-6]. Mitochondrial changes include dysfunction of the oxidative phosphorylation (OXPHOS) as well as mitochondrial-related gene expression. Mitochondrial abnormalities in the genome were found in SZ [7] and BD [8]; expression of mitochondria-related genes was altered in post mortem brains of patients with BD or SZ. Other evidence comes from studies using positron emission tomography (PET), magnetic resonance spectroscopy (MRS), single photon emission computer tomography (SPECT) and proton echo-planar spectroscopic imaging (PEPSI).

Conversely, mitochondrial diseases are frequently comorbid with psychotic symptoms and misdiagnosed BD or SZ [9]. The hypothesis of mitochondrial variants in SZ, BD and MDD is supported by the inheritance of mitochondrial DNA (mtDNA). mtDNA is matrilineal; higher rates of maternal offspring with mental diseases were found in comparison to paternal rates. This was observed in family studies, rather than in the general population, thus lending support to the hypothesis that the origin of these diseases can be related to mitochondrial dysfunctions.

Impaired functions of mitochondria can be assessed both in isolated mitochondria and in intact or permeabilized cells. A better insight into molecular mechanisms of cellular respiration, control of oxidative phosphorylation (OXPHOS) and effects of psychotropic drugs on these processes is likely to lead to a better understanding of the pathophysiology of neuropsychiatric disorders.

1.1 Depression and hypotheses of depression

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.

Recently, attention in the research of the 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 [10], e.g. to changes in neuroprogression, inflammatory and cell-mediated immune response, antioxidant capacity, oxidative and nitrosative stress, and mitochondrial functions [11]. Therefore, changes in the activities of compounds of these intracellular signaling pathways are studied with the aim of discovering new biological markers of mood disorders or predictors of response to antidepressant treatment [12, 13]. Mitochondrial dysfunctions are assuming an increasingly important role in hypotheses of mood disorders, mainly BD. Recently discussed biological hypotheses of mood disorders include the neurotrophic and the neuroplasticity hypothesis of depression [10, 14-17], and the mitochondrial hypothesis [18-20].

Research findings about intracellular processes associated with mood disorders and long-term effects of antidepressants demonstrate an important role for signaling pathways primarily regulated by monoamine neurotransmitters; this has been settled as the basis of many biochemical hypotheses [21, 22]. While dysfunctions within monoaminergic neurotransmitter systems are likely to play an important role in the pathophysiology of mood disorders, this 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 propose that mood disorders are caused by structural or functional changes in molecules and signaling 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 the 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 rhythms, and mitochondrial dysfunctions [11, 23-25].

Research into the biological basis of mood disorders emphasizes changes in 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 [10]. 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 BD [20].

The monoamine hypothesis

The discovery of the first effective antidepressants - MAO (monoamine oxidase) inhibitors and tricyclic antidepressants - prompted the hypothesis of a significant role for the biogenic amine, particularly NE and serotonin in the etiopathogenesis of affective disorders. The classic monoamine hypothesis is an early milestone in the field of depression. It proposed that depression might be produced by a serotonin or NE deficiency at functionally important receptor sites in the brain, i.e. that brain monoamine systems have a primary direct role in depression [26, 27]. Soon it became evident that the monoamine hypothesis in its original form could not explain all of the effects of antidepressants [28, 29]. In order to test this hypothesis, a series of studies was conducted to evaluate the effects of monoamine depletion on depressive symptoms in depressed patients and in healthy controls. Relapse to serotonin depletion or to catecholamine depletion was found to be specific to the type of antidepressant treatment and type of depletion. Serotonin or NE/dopamine depletion did not decrease mood in healthy controls and slightly lowered mood in healthy controls with a family history of MDD. In drug-free patients with MDD in remission, a moderate mood decrease was found only for acute tryptophan depletion. However, acute tryptophan depletion induced relapse in patients in remission who used serotonergic antidepressants [30]. Depletion studies failed to demonstrate a causal relation between serotonin or NE and depressive disorder [31, 32]. The effects of acute tryptophan depletion on cognition in non-vulnerable participants are independent of mood changes [33]. Even simultaneous disruption of serotonin and catecholamine systems did not significantly alter mood in untreated depressed subjects [34]. These findings forced a major revision of the classic monoamine hypothesis of depression. According to this revised monoamine theory of depression

[25, 35], monoamine systems only modulate other brain neurobiological systems that have a more primary role in depression.

The neurotrophic hypothesis

The neurotrophic hypothesis of depression [14-17] proposed 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 the transcription factor CREB (cAMP response element binding protein), higher expression of the neurotrophin BDNF and its receptor trkB, and consequently to increased neuronal plasticity and resumption of cellular functions.

According to the neurogenic hypothesis [36, 37], depression may develop due to the decreased neurogenesis in the hippocampus, and antidepressants takes effect through the stimulation of neurogenesis. The cellular plasticity hypothesis [38] relates the neurotrophic and the neurogenic hypotheses to the statement that depression can be generally caused by damaged cellular plasticity leading to inadequate relations between structure and function. The molecular mechanisms, which lead to a disturbance of neuroplasticity, are not known. The bioenergetic and neurochemical model of BD attempts to identify these mechanisms and focuses attention on mitochondrial dysfunctions [18, 39].

The mitochondrial hypothesis

The relationship between mitochondrial dysfunction and unipolar depression was explored in several studies [1, 40, 41]. It was demonstrated that muscle mitochondria in depressed patients produced less ATP and activity of complexes I + III and II + III was impaired [42]. It was proposed that energy depletion constitutes at least part of the inherited biological predisposition towards the development of depression with somatization [43]. Level of complex I subunit (NDUFS7) and complex I activity were found decreased in MDD patients [44].

The inflammatory and neurodegenerative hypothesis

The central nervous system, endocrine and immune systems use neurotransmitters, cytokines and hormones to communicate among themselves [45]. Now there is evidence that activation of the immune system is associated with the symptoms of depression [46, 47]. The inflammatory and neurodegenerative hypothesis of depression [48] proposes that depression is associated with inflammatory processes, along 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 Bipolar disorder and hypotheses of bipolar disorder

BD is one of the psychiatric disorders leading to substantial impairment in psychosocial function, the sixth leading cause of disability worldwide. BD features intermittent episodes of mania or hypomania and depressive episodes; rapid cycling; mixed states; and, in some cases, psychotic symptoms. Patients with BD very often experience periods of depression, hypomania or mania, even if the best available treatment is used. Residual symptoms of this illness persist many times between episodes [49]. Depression and mania are thought to be heterogeneous illnesses that can result from dysfunction of several neurotransmitters or metabolic systems. BD still has low recovery rates and high rates of treatment-resistant cases; about one-third of BD patients admit to at least one suicide attempt. Relative to the general population, the rates of disability and premature mortality are two to three times higher [50]. The pathophysiology of BD is complex, multifactorial, and not fully understood. Insights into the pathophysiologic processes underlying BD have been provided by studies examining structural and functional changes in the brain. Damage in neuronal circuits, impairment in neuronal plasticity and resilience, disturbances of synaptic transmission and signal transduction caused by or associated with oxidative and nitrosative stress, neurotrophins, mitochondrial dysfunctions, neuroinflammation, autoimmune

processes, tryptophan and tryptophan metabolites, and HPA axis dysregulation were reported [51]. BD is characterized by multiple associations between disturbed brain development, neuroplasticity, and chronobiology, caused by genetic and environmental factors, and defects in apoptotic, immune/inflammatory, neurotransmitters, neurotrophins, and calcium signaling pathways, oxidative and nitrosative stress, cellular bioenergetics, and membrane or vesicular transport. There is a growing amount of evidence for the association of mitochondrial dysfunction with psychiatric illnesses both *in vitro* and *in vivo*.

The monoamine hypotheses

The monoamine hypothesis was initially formulated as catecholamine [27] and/or indolamine [26] deficiencies in the brain; i.e. as the neurotransmitter hypothesis. Later, the monoamine hypothesis was revised to include the role of neurotransmitter receptors, transporters, catabolizing enzymes (monoamine oxidase, MAO, and catechol-*O*-methyltransferase, COMT), and other brain neurobiological systems [25, 35, 52, 53].

The monoamine hypothesis of mood disorder posits that an imbalance in monoaminergic neurotransmission in the central nervous system is causally related to the clinical features of depression or mania. This hypothesis is supported by the mechanisms of action of antidepressants [54, 55]. Moreover, many candidate genes associated with BD encode compounds directly influencing directly the monoamine neurotransmitter systems, e.g., *SLC6A4* (encoding serotonin transporter, SERT, 5-HTT), *TPH2*, *DRD4*, *SLC6A3I* (encoding dopamine transporter), *MAOA* (encoding MAO-A) and *COMT* (encoding COMT) [56-58].

The advanced monoamine hypothesis [59] proposes that serotonin or norepinephrine (NE) concentrations in the brain are regulated by MAO-A activity, and the severity of symptoms of depression is linked to changes in the activity of monoamine transporters in specific brain regions. The hypothesis has been supported by observation of elevated MAO-A density and reduced SERT density during depressive episode [59-61].

The mitochondrial hypothesis

MtDNA mutations in the brain, associations of mtDNA polymorphisms with BD and changes in gene expression related to mitochondria in the brain have been observed [9, 19]. The mitochondrial dysfunction hypothesis of BD is based on these observations. According to this hypothesis, mtDNA polymorphisms/mutations or mtDNA deletions caused by nuclear gene mutations can cause mitochondrial dysregulation of calcium leading to symptoms of BD [19, 39, 62]. The mitochondrial hypothesis corresponds to the above mentioned, neurotrophic hypothesis because of the important role of the calcium signaling pathway in synaptic plasticity regulation.

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

The role of mitochondrial dysfunction during BD is supported both by observation of the changes in brain metabolism and by the effects of mood stabilizers (lithium and valproate) on mitochondrial functions. Metabolic changes in brain have been observed in BD by MRS. This suggests the suppositions that mitochondrial dysfunctions include impaired OXPHOS, a final shift to glycolytic production of energy, a general decrease of energy (decreased ATP production), changed concentrations of phosphomonoesters and changed lipid metabolism [18].

Other BD hypotheses

Hyperdopaminergic function has been reported in BD. A dopamine hypothesis of BD was formulated that suggests a role for increased dopaminergic transmission in mania and the converse in depression [63, 64]. The hypothesis is supported by the fact that altered availability of dopamine transporter has been accepted as a biomarker for BD [61].

Monoamine depletion studies, genetic association studies, PET studies, and the mechanism of action of antidepressants supported an important role for disturbed monoamine neurotransmission in the pathophysiology of mood disorders but have not evidenced the primary role of the monoaminergic system in the development of the disorder. The molecular changes underlying neurotransmission imbalances in BD are not agreed upon; it is hypothesized that alterations in

excitatory amino acid transporters, SERT, and dopamine transporter contribute to altered glutamatergic and monoaminergic function in BD patients [65].

The hypothesis that a dysfunctional muscarinic acetylcholine system is involved in the pathophysiology of BD has been supported by the finding that there is reduced muscarinic acetylcholine M_2 receptor binding in subjects with BD [66], which could be accounted by a reduction in M_2 receptor affinity caused by genetic variation in the gene for the M_2 receptor [67].

Glutamate levels were increased in post-mortem brain of subjects with BD, while the glutamate/glutamine ratio was decreased following valproate treatment, and gamma-aminobutyric acid (GABA) levels were increased after lithium treatment. The balance of excitatory/inhibitory neurotransmission seems to be central to the BD [68]. A meta-analysis confirmed that brain glutamate + glutamine levels are elevated in BD patients [69], which supported an important role of glutamate in the pathophysiology of BD. Due to the role of glutamate in neurotransmission, brain energy metabolism, astrocyte function, neurotoxicity, neuroplasticity, and learning, the glutamate hypothesis of mood disorders is expected to complement and improve upon the prevailing monoamine hypothesis [70, 71]. The hypothesis is supported by observation that antagonists of glutamate *N*-methyl-D-aspartate (NMDA) receptor produce a rapid antidepressant effect [72, 73].

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. BD).

The antidepressant activity of the first generation of antidepressants, tricyclic antidepressants and MAO inhibitors, 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 [74].

Although a wide range of pharmacologically different antidepressants and mood stabilizers is available, molecular mechanisms of their therapeutic effects have not 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 [75].

Effects of antidepressants on mitochondrial functions are summarized in the Table 1.

Table 1 Effects of antidepressants on mitochondrial functions

Antidepressant	**Biological model**	**Affected mitochondrial function**	**Ref.**
Imipramine	Isolated rat liver mitochondria Beef heart submitochondrial particles	Uncoupling effects on OXPHOS (release of respiratory control, hindered ATP synthesis, enhanced ATP synthase activity) Inhibition NADH oxidation, inhibition of ATP synthase	[76]
Imipramine	Rat brain mitochondria	Increased state 3 and state 4 respiratory rates	[77]
Imipramine	Rat liver mitochondria	Increased state 3 and state 4 respiratory rates	[78]
Imipramine, clomipramine, citalopram	Human peripheral lymphocytes and lymphoblasts	Dose-dependent induction of apoptosis	[79] [80]
Imipramine, clomipramine, citalopram	Human acute myeloid leukemia HL-60 cells	Loss in cell viability, increased ROS production, loss of $\Delta\psi_m$	[81]
Clomipramine, desipramine, norfluoxetine, tianeptine	Rat heart isolated mitochondria CHO cells	Reductions of $\Delta\psi_m$, Decrease in state 3 respiration Inhibition of activities of complexes I, II/III and IV Insignificant change of $\Delta\psi_m$, decrease in state 3 respiration Inhibition of complex I activity	[82]
Tianeptine	Rat liver mitochondria	Inhibited beta-oxidation and TCA cycle	[83]
Fluoxetine	Rat liver mitochondria	Inhibition of state 3 respiration, stimulation of state 4 respiration, decrease of respiratory control ratio and uncoupling effects on OXPHOS	[84]

Antidepressant	Biological model	Affected mitochondrial function	Ref.
Fluoxetine	Rat brain mitochondria	Inhibition of OXPHOS, decreased activity of ATP synthase	[85]
Amitriptyline, fluoxetine	Differentiated rat pheochromocytoma PC12 cells	Prevention of the loss of $\Delta\psi_m$, cyt *c* release, formation of ROS induced by MPP^+	[86]
Amitriptyline, fluoxetine	Rat pheochromocytoma cells	Attenuation of H_2O_2 neurotoxic effects, upregulation of superoxide dismutase	[87]
Nortriptyline	ALS mouse	Strong inhibitor of MPT	[88]
Nortriptyline	Mouse model of ischemia	Inhibition of $\Delta\psi_m$, inhibited release of mitochondrial factors and caspase 3 activation	[89]
Nortriptyline	Rat brain mitochondria	Inhibitor of MPT, inhibition of ETC, mild uncoupling	[90]
Fluoxetine and/or olanzapine	Rat brain homogenates	Increased CS activity after acute, but not chronic treatment	[91]
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	[92]
Nefazodone Trazodone	Isolated rat liver mitochondria	Complex I and complex IV inhibitor No effect	[93]
Fluoxetine	Hippocampal synaptic plasma membranes	Increased ATP synthase activity	[94]
Sertraline	Isolated rat liver mitochondria	Uncoupling effects on OXPHOS, inhibition of complex I and complex V activities, induction of Ca^{2+} mediated MPT	[95]
Venlafaxine, paroxetine, nortriptyline	Rat brain homogenates (after 15 days of drug administration)	Differences in brain areas: increased or unchanged CS and SDH activities	[96]
Paroxetine, fluoxetine, clomipramine	Rat glioma and human neuroblastoma cell lines	Increased cyt *c* release, caspase-3-like activity, induction of apoptosis	[97]
Desipramine	Rat glioma cells	Activation of caspases 3 and 9, no changes of $\Delta\psi_m$	[98]

List of abbreviations: ALS mouse - amyotrophic lateral sclerosis mouse (a model of neurodegeneration), ATP - adenosine 5´-triphosphate, CHO cells - Chinese hamster ovary cell line, CS - citrate synthase, cyt *c* - cytochrome *c*, ETC - electron transport chain, H_2O_2 - hydrogen peroxide, OXPHOS - oxidative phosphorylation, MPP^+ - methyl-4-phenylpyridinium, MPT - mitochondrial permeability transition, NADH - reduced nicotinamide adenine dinucleotide, ROS - reactive oxygen species, SDH - succinate dehydrogenase, TCA - tricarboxylic acid, $\Delta\psi_m$ - mitochondrial membrane potential

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.

Mood stabilizers affect multiple sites in intracellular signaling pathways [13]. Main targets of mood stabilizers are neurotrophin BDNF, extracellular signal regulated kinase-1 (ERK) pathway, and pathways modulated by glycogen synthase kinase 3 (GSK-3) or Bcl-2 [17, 99, 100]. 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 signaling pathways regulated by multiple drugs, e.g. protein kinase C, 3´,5´-cyclic adenosine monophosphate (cAMP), arachidonic acid, etc. [101]. Furthermore, lithium and valproate reduce transport of myo-inositol into the cells, which leads to reduced protein kinase C activity. Lithium and valproate increase Bcl-2 concentrations [102] and inhibit GSK-3 activity (lithium directly, valproate indirectly). Valproate activates mitogen-activated protein kinase (MAPK) signaling pathway and regulates stress proteins of endoplasmic reticulum [103]. Through the effects on Bcl-2 and p53 (proapoptotic protein), lithium affects mitochondria by stabilization of membrane integrity and prevention of mitochondrial permeability transition pores (mPTPs) opening; i.e. by regulating the key process in cell death leading to at least temporary loss of inner membrane potential ($\Delta\psi_m$), 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 [104]. Increased concentrations of *N*-acetylaspartate (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 MAO inhibitors and inhibitors of the reuptake of serotonin and/or NE [105-107]. The mode of action for the

lithium augmentation of antidepressants is partly mediated by an increase of 5-HT neurotransmission [108-110]. However, lithium could not either inhibit MAO-A or MAO-B in the brain mitochondrial [111, 112]. 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 [113].

Studies have shown effects of mood stabilizing drugs on mitochondria. In isolated brain mitochondria lithium caused desensitization to calcium, antagonized permeability transition, and diminished cytochrome *c* (cyt *c*) release [114]. In isolated rat liver mitochondria valproate inhibited OXPHOS [115]. In isolated pig brain mitochondria both lithium and valproate inhibited respiratory chain complexes I and IV [116]. According to study performed in rats [117], 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 [118]. Chronic treatment of SH-SY5Y cells prevents reduction of methamphetamine-induced reduction of cyt *c*, mitochondrial antiapoptotic Bcl-2/Bax ratio and mitochondrial (complex IV, COX) activity [119]. Interestingly, long-term lithium and valproate did not protect SH-SY5Y cells against endoplasmic reticulum stress-induced cytotoxicity [118]. Lithium and carbamazepine could facilitate activation of CREB, valproate and lamotrigine did not affect BDNF-mediated signaling [120]. Thus, these mood stabilizers likely decrease the vulnerability of mitochondrial functions caused by oxidative stress and have neuroprotective effects [118].

Chronic treatment with lithium, valproate and carbamazepine protects against NMDA-mediated toxicity [121]. 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 [122]. Carbamazepine interferes in adenylate cyclase pathway: inhibits adenylate cyclase and the synthesis of cAMP [123]. Lamotrigine prevented the toxicity caused by rotenone and MPP^+ in rat PC12 cells by suppressing the MPT formation, which leads to cyt *c* release and subsequent apoptosis. Though, lamotrigine seems to have neuroprotective effect due to the mitochondrial respiratory complex I inhibition [124].

Effects of mood stabilizers on mitochondrial functions are summarized in the Table 2.

Table 2 Effects of mood stabilizers on mitochondrial functions

Mood stabilizer	Biological model	Affected mitochondrial function	Ref.
Valproate	Rat liver mitochondrial fractions	Inhibition of the rate of oxygen consumption, sequestration of intramitochondrial coenzyme A	[125]
Valproate	Isolated rat liver mitochondria	State 3 rates of oxygen consumption inhibited	[115]
Valproate	Isolated beef brain α-ketoglutarate dehydrogenase	Inactivation of α-ketoglutarate dehydrogenase complex	[126]
Valproate and its metabolites	Submitochondrial particles prepared from rat liver	Inhibition of pyruvate uptake	[127]
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)	[128]
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	[129]
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	[117]
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)	[130]
Valproate and lithium	Human neuroblastoma and glioma cells	Protective effects against H_2O_2 or rotenone induced cytotoxicity in neuroblastoma cells	[118]
Valproate and lithium	Human neuroblastoma cells	Reduction of methamphetamine-induced reduction of cyt *c*, antiapoptotic Bcl-2/Bax ratio and COX activity	[119]
Lithium	Plasma synaptic membrane from rat brain (as an animal model of depression)	Impaired function of ATP synthase was modulated (reversed by lithium treatment, prevented by lithium pretreatment)	[130]
Lithium	Isolated brain mitochondria	Desensitization to calcium, antagonized MPT, diminished cyt *c* release	[114]
Lithium	Postmortem human brain cortex	Dose-dependent increased activities of complexes I+III, II+III and SDH	[131]
Lithium	Human neuroblastoma SH-SY5Y cells	Attenuation of rotenone-induced caspase-3 activation	[132]
Carbamazepine	Rat liver mitochondria	Decreased state 3 respiration, respiratory control ratio, ATP synthesis, $\Delta\psi_m$	[133]

Mood stabilizer	Biological model	Affected mitochondrial function	Ref.
Carbamazepine	Rat brain mitochondria	Protection against rotenone induced complex I inhibition	[134]
Carbamazepine, lamotrigine	Human white blood cells	Carbamazepine decreased ATP production, stimulatory effect on production by lamotrigine	[122]
Lamotrigine	Human neuroblastoma SH-SY5Y cells	Suppression of MPT formation, attenuation of rotenone-toxicity, inhibition of ROS production	[124]

List of abbreviations: ATP - adenosine 5′-triphosphate, Bax - Bcl2 associated X, apoptosis regulator, Bcl-2 - B-cell CLL/lymphoma 2, COX - cytochrome *c* oxidase, cyt *c* - cytochrome *c*, H_2O_2 - hydrogen peroxide, MPT - mitochondrial permeability transition, ROS - reactive oxygen species, SDH - succinate dehydrogenase, SH-SY5Y - human neuroblastoma cells, $\Delta\psi_m$ - mitochondrial membrane potential

1.4 Inhibition of MAO

Monoamine oxidase (MAO; E.C. 1.4.3.4) is an enzyme located on the outer mitochondrial membrane and is responsible for the oxidation of both biogenic and xenobiotic monoamines. There are two isoforms of MAO differing in substrate preference, inhibitor specificity, tissue and cell distribution, and immunological properties. MAO-A isoform metabolizes serotonin, whereas MAO-B preferentially metabolizes 2-phenylethylamine or benzylamine as substrates. MAO-B is localized predominantly in serotonergic neurons and astrocytes. Both isoforms are present at high concentrations in the brain with the purpose of keeping monoamine concentrations in cytosol very low. MAO inhibition is the best known direct action of some antidepressants on mitochondrial enzymes.

The antidepressant effect of MAO inhibitors 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 [135]. It is known to act as a pro-drug and can be converted into isopropyl hydrazine which binds covalently to MAO [136]. 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 (PD). Inhibitors of MAO lose their selectivity at high doses. Moreover, there are feedbacks and interconnections of intracellular signaling pathways, which lead to mutual interactions of monoaminergic and other systems [137, 138]. Thus, inhibiting of MAO-B should influence processes mediated primarily by substrates for MAO-A. The major disadvantage was the incidence of cheese reaction with irreversible inhibitors [139].

The selective reversible MAO-A inhibitors such as moclobemide increase the content of serotonin, NE and dopamine in the brain [140], but did not provoke the cheese reaction. Moclobemide was 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 [105, 141-146]. 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 [147]. However, major metabolites in plasma were less effective MAO-A inhibitors than moclobemide or they were pharmacologically inactive [148, 149].

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 [150]. Antidepressants, which act primarily as serotonin and/or NE reuptake inhibitors, show also inhibitory activity towards MAO. It has been suggested that tricyclic antidepressants exert some of their therapeutic effect by inhibiting MAO [151]. They are able to inhibit MAO-B both *in vitro* [152, 153] and *in vivo* [154, 155]. However, *in vivo* inhibition of the human platelet MAO-B in the patients taking tricyclic antidepressants was not confirmed by others [156, 157]. Amitriptyline, clomipramine, desipramine, imipramine and iprindole have comparable potencies as inhibitors of MAO in rodent brain and liver [158]. These antidepressants have been shown to partially protect mouse brain MAO *in vivo* from the irreversible enzyme inhibition produced by subsequent injection of phenelzine [158]. Concentrations of tricyclic antidepressants, showing a pronounced inhibitory effect on MAOs activity, were significantly higher than plasma levels of the drug found under therapeutic conditions [111, 159]. MAO activity was inhibited after long-term administration of zimelidine, maprotiline, imipramine, amitriptyline, and nortriptyline in systematic studies of Egashira [160, 161]. 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 [162]. Fluoxetine and norfluoxetine showed affinities for both MAO-A [163] and MAO-B [164]. 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.

2. Mitochondria and Alzheimer's disease

Alzheimer's disease (AD) is the most common neurodegenerative disorder marked by progressive loss of memory and impairment of cognitive ability. AD can be classified into two forms: sporadic AD, where aging represents the main risk factor, in the vast majority of cases; and familial form of AD, where rare gene mutations have been identified [165, 166]. Both sporadic and familial AD patients share common clinical and neuropathological features including loss of neurons, intracellular neurofibrillary tangles (aggregates of hyper-phosphorylated tau protein), and extracellular senile plaques, composed of amyloid β (Aβ) deposits, which are derived from the proteolytic processing of the amyloid precursor protein (APP) [167]. According to a body of evidence, Aβ increases the neuron vulnerability to oxidative stress and impairments of ETC [168]. Pathologically, AD is featured by changes observed mostly in neocortex, hippocampus, and other subcortical regions essential for cognitive functions. Reduction in a variety of higher cortical functions – memory, orientation, and judgment is evident [4].

MAO activities were found to be increased in the brains of AD patients [169, 170]. MAO is involved in neurodegenerative processes via elevation of hydrogen peroxide (H_2O_2) production and oxidative stress [171]; therefore, monoamine neuromodulators (such as NE, dopamine, and serotonin) have increased turnover [170]. Activation of MAO increased β-secretase and γ-secretase expression, further improving Aβ formation from APP [170]. Activation of MAO may also be connected with the generation of neurofibrillary tangles. A lot of drugs cause as MAO inhibitors. Some of them play a role in antidepressive and neurodegenerative therapy, such as PD and AD disorders [112, 172]. MAO inhibitors decrease catabolism of monoamine neuromodulators and lower generation of ROS. MAO inhibitors advance cognitive improvement, modulate proteolytic cleavage of APP, which leads to decrease of pathogenic Aβ. Therefore, MAO inhibitors are considered to be multitarget drugs, recently developing for AD treatment [170].

Besides the presence of the pathological proteins Aβ and tau protein, a pathophysiologic decrease of cholinergic neurotransmission dominates in AD. Damage of the system of

acetylcholine neurotransmission in early stages of AD contributes to cognitive impairment, loss of attention and behavior disorders [173]. ChE inhibitors attenuate the cholinergic deficit and have positive effects on cognition and behaviour of AD patients [174].

Acetylcholine is cleaved by two cholinesterases: acetylcholinesterase (AChE, E.C. 3.1.1.7), butyrylcholinesterase (BChE, E.C. 3.1.1.8). AChE and BChE vary in their expression, specificity, and activity in various areas of the CNS. Changes in ChE activities occur during the human lifecycle and in some pathological conditions; in the case of AD, the activity of AChE diminishes while BChE activity increases [175]. AChE is occurred primarily in neurons, in G1 and G4 isoforms. For AD is typical selective loss of the AChE G4 isoform; a mild grow in the G1 isoform of AChE and BChE was also noticed [176]. BChE activity rises in the AD brain, whereas AChE stays unaltered or decreases [177]. Therefore, both AChE and BChE are a crucial goal in AD therapy [177, 178].

2.1 Mitochondrial involvement in neurodegenerative diseases

The series of events that lead to neurodegeneration are intricate. Various neurodegenerative disorders manifest with different symptoms and affect different parts of the brain. Mitochondrial dysfunctions are considered as conjunctive features, a point of convergence to different pathological pathways.

The mitochondria are cytoplasmic organelles in eukaryotic cells that are responsible for most of energy supply of cells. Besides, they are critical regulators of cell death, a key feature of neurodegeneration [179], and play important role in cell processes, signaling pathways, calcium homeostasis, cell cycle regulation, apoptosis, reactive oxygen species (ROS) production and thermogenesis [180]. The mitochondrial dysfunction, increased ROS production and oxidative damage are responsible for numerous neurodegenerative disorders. Apoptosis and excitotoxicity are the two significant grounds of neuronal cell death and the role of mitochondria is crucial in both the cases [181]. Increased ROS production in neurodegenerative process might affect mitochondrial parameters also: ATP production, membrane potential, mPTP activation, and calcium uptake. These changes can lead and result in neuronal damage. The first evidence of involvement of mitochondria in pathogenesis of neurodegenerative process was reported when complex I deficiency was detected in substantia nigra and platelet mitochondria of patients with

PD [182, 183]. Further strong evidences were found for ETC deficiencies: complex I and COX in AD and complex II and III in Huntington's disease [184].

Biochemical analysis of postmortem AD brains found impaired function of the citric acid cycle enzymes, pyruvate dehydrogenase, α-ketoglutarate dehydrogenase and isocitrate dehydrogenase. These changes correlated with the clinical state, the function of enzymes could be related to diminished brain metabolism [185].

2.2 Mitochondrial ROS and apoptosis

The imbalance between cellular production of ROS and the ability of cells to efficiently defend against them is called 'oxidative stress.' Oxidative stress is linked to neurodegenerative diseases and aging processes, it can be the source of cellular damage causing necrotic or apoptotic cell death since the ROS oxidize vital cellular components, lipids, proteins and nucleic acids [186].

Impaired function of oxidative phosphorylation (OXPHOS) may cause disturbances of energy metabolism, which are frequently observed in AD. Impaired energy metabolism results to decreased respiratory control ratio as well as ATP levels [187]. There are many possible mechanisms for reduced oxidation rates and ATP production rates that do not include a defect of respiratory chain enzymes [188].

ROS have their role in intracellular signaling and regulation of signal transduction [166]. ROS seem to be the key factors in brain aging processes and disturbed mitochondrial respiration, accompanied by increased ROS production, significantly contributes to functional changes in brain during aging. Complex I and complex III are considered to be the primary source of ROS in brain under physiological conditions, as well as in pathological processes (e.g. neurodegenerative disorders). Complex I releases superoxide to matrix, complex III can release superoxide to both sides of the inner mitochondrial membrane. Superoxide dismutase can convert superoxide to H_2O_2, which permeate by membranes and can be source of highly reactive hydroxyl radical. Physiologically generated H_2O_2 and superoxide from ETC are dependent on the magnitude of proton-motive force and the respiratory state of mitochondria [189]. State 4 is characterized with high rate of ROS, contrary, states 3 and 5 produce minimum of ROS [190].

Both disturbed production and detoxification of ROS participate on pathophysiological effects of mitochondrial dysfunctions [11, 191]. Defective mitochondria release large amounts of ROS, similarly, decline of antioxidative enzyme activities (e.g. in elderly) enhances ROS formation [192]. Negative results of ROS can affect respiratory chain: complexes I, III and COX seem to be the most affected, whereas function of complex II appears to be unchanged [193, 194].

Mitochondria play a pivotal role in intrinsic pathway of apoptosis [195]. During apoptosis, mitochondrial network is disintegrated and the outer mitochondrial membrane is permeabilized, which leads to release of several apoptotic proteins, cyt *c* included. There are interrelated mitochondrial pathways that facilitate cell death: (i) opening of mPTPs can lead to mitochondrial swelling and cell death through apoptosis or necrosis; (ii) increase in the permeability of the mitochondrial membrane causes leak of apoptotic factors (second mitochondria-derived activator of caspases (Smac) and cyt *c*, which trigger the caspase cascade leading to apoptosis); and (iii) release of caspase-independent death effector, apoptosis-inducing factor (AIF), triggers chromatin condensation and DNA degradation [196]. Mitochondria undergo fragmentation during apoptosis before caspases are activated [197]. In apoptotic cells rapid loss of the inner mitochondrial membrane potential ($\Delta\psi_m$) is accompanied by ROS production.

Recently, attention is paid to the ROS-induced damage of ETC complexes mediated by a peroxidation and oxidative damage of cardiolipin [198-200]. Membrane lipids, cardiolipin mainly, are both required for the stability of respiratory supercomplexes and serve as a diffusion microdomain for the ubiquinone [201]. Cardiolipin plays also an active role in mitochondrial mediated apoptosis, can be oxidized, and interacts with cyt *c* and Bcl-2 proteins [202].

In AD, membrane-associated oxidative stress, increased free radical production and perturbed Ca^{2+} homeostasis have been observed. Increased mitochondrial permeability and cyt *c* release, which is promoted by Aβ and alpha-synuclein oligomerization and polymerization, trigger the opening of mPTP leading to apoptosis [203]. In addition, COX activity is reduced and neurons exhibit mitochondrial damage and apoptosis. However, the cause of mitochondrial alterations in AD remains unknown.

2.3 Impairment of ETC in Alzheimer's disease

Activity of COX was found to be reduced in platelets of AD patients [204]. Similarly, significantly decreased COX activity was observed in cortex of AD patients [205]. Another study confirmed the decreased activity in hippocampus of AD patients that suggests the anatomical specificity [206]. Mitochondrial deficiencies were found in platelets of AD patients indicating significant decline of complex III and COX activity [207]. It has been shown that AChE was reduced, further, it was demonstrated that AChE could increase the Aβ activity [208, 209].

ETC activities of human lymphocytes were evaluated in AD patients, increased complex II and IV activities were observed; this might be a compensatory mechanism to supply the energy [210]. Evidences of ETC dysfunctions in AD are summarized in Table 3.

Distinct mitochondrial abnormalities associated with neurodegenerative diseases culminate in oxidative stress, energy dysfunction, and aberrant homeostasis of cytosolic calcium [211]. System of OXPHOS does not respond to thermodynamic equilibrium, but embodies a rate of uncoupling. Lower $\Delta\psi_m$ can result in hydrolysis of cytoplasmic ATP; high $\Delta\psi_m$ leads to proton leak and increased uncoupling. ROS overproduction, decreased $\Delta\psi_m$ and Ca^{2+} dependent increase of MPT lead to apoptosis [190]. Decreased rates of electron transfer were identified as mechanism of mitochondrial dysfunction on aging, complex I and COX were found decreased upon aging [212]. Inhibition of complex III and COX is required to increase glutamate release Ca^{2+} independent [213]. Partial inhibition of complex I activity reduced nerve terminal oxygen consumption and increased glutamate release from depolarized synaptosomes [214].

Table 3 Evidences of ETC dysfunctions in AD

Biological model	Affected mitochondrial function	Reference
Lymphocyte mitochondria of AD patients	Higher oxidative (oxidation of pyruvate - malate, glycerol-3-phosphate) and enzymatic activities (I, II and III) were found in AD patients treated with rivastigmine than untreated AD patients	[215]
Transgenic mice crude forebrain	Tau dependent deregulation of complex I and Aβ dependent deregulation of complex II, synergistic effects of deregulation in AD mice, reduction in mitochondrial membrane potential	[216]
Lymphocytes	Alterations in respiratory chains: activity of complex II and IV was higher	[210]
Platelets and postmortem motor	COX but not F_0F_1-ATPase is a mitochondrial target in AD, in	[217]

Biological model	Affected mitochondrial function	Reference
cortex and hippocampus from AD patients	both a brain association area and in platelets. A reduced COX activity may make the tissue vulnerable to excitotoxicity or reduced oxygen availability	
Posterior cingulate (area 23) cortex	The findings suggest a decrement of COX in posterior cingulate cortex, with progressive reduction within the superficial laminas linked to disease duration	[218]
Platelet and lymphocyte mitochondria	Significant declines in Complex III and COX	[207]
Postmortem brain tissue	Complex I, complex II-III slightly decreased in occipital cortex, COX decreased significantly in cortical areas (frontal, temporal, parietal, occipital)	[205]
Autopsied human brain mitochondria	AD brain mitochondria demonstrated a generalized depression of activity of all ETC complexes. This depression was most marked in COX activity ($p < 0.001$). Concentrations of cytochromes b, c1, and aa3 were similar in AD and controls. The ETC is defective in AD brain, and the defect centers about COX	[219]
Subcortical centers: thalamus, the globus pallidus, the red nucleus, and the locus coeruleus	Changes of the mitochondrial cristae, accumulation of osmiophilic material and decrease of their size, mitochondrial alterations were particularly prominent in neurons, which showed loss of dendritic spines and abbreviation of the dendritic arborization	[220]
Human seven brain regions (cerebellum, frontal, temporal, occipital, parietal cortices, thalamus and caudate nucleus)	Complex III core protein was significantly reduced in the temporal cortex of AD patients	[221]
Autopsied brain mitochondria	COX activity reduced in frontal, temporal and parietal cortices, normal COX activity in occipital cortex	[222]
Human seven brain regions (cerebellum, frontal, temporal, occipital, parietal cortices, thalamus and caudate nucleus)	Complex I 24-kDa subunit was significantly reduced in temporal and occipital cortices. Complex I 75-kDa subunit was significantly reduced in parietal cortex region of brain	[223]
Human brain: frontal cortex, temporal cortex, hippocampus, and cerebellum	Specific defect of COX in the confined brain regions, suggesting anatomic specificity	[206]
Human cytoplasmic hybrid (cybrid) neurons with incorporated platelet mitochondria	Significant changes in morphology and function; such changes associate with altered expression and distribution of Dlp1 and Mfn2, mitochondrial fission-fusion imbalances	[224]
In situ nerve terminal and synaptosomal mitochondria of rats	High level of inhibition is required for glutamate efflux from nerve terminal	[213]
Rat forebrain mitochondria	Loss of cyt *c* by mitochondria oxidizing NAD^+-linked substrates results in a dramatic increase of ROS production and respiratory inhibition	[225]
Mitochondria from brains of transgenic mice	Aβ progressively accumulates in mitochondria and is associated with diminished enzymatic activity of complex III	[226]

Biological model	Affected mitochondrial function	Reference
	and COX, reduction in the rate of oxygen consumption	
Human neuroblastoma cells (SH-SY5Y)	Increased complex III activity, decreased COX activity were found Decreased respiratory control ratio and ATP levels	[39]
Human blood platelets	ATP levels were reduced, while ROS were increased in AD patients. Platelet membrane fluidity, vitamin E, and cholesterol content were similar between effected and non-effected groups	[227]

List of abbreviations: Aβ – β-amyloid, AD – Alzheimer's disease, ATP - adenosine 5′-triphosphate, Bcl-2 - B-cell CLL/lymphoma 2, COX - cytochrome *c* oxidase, , cyt *c* - cytochrome *c*, Dlp1 - dynamin-like protein 1, Mfn2 - mitofusin 2, NAD - nicotinamide adenine dinucleotide, ROS - reactive oxygen species

2.5 Clinically used drugs for AD treatment and mitochondrial functions

Donepezil is a non-competitive and reversible AChE inhibitor. Donepezil attenuated the mitochondrial dysfunctions, decreased mitochondrial calcium and reversed the $\Delta\psi_m$ induced by okadaic acid in rat brain mitochondria [228]. Neuroprotective effect of ChE inhibitors was observed in neuroblastoma SH-SY5Y cells against okadaic acid or $A\beta_{25-35}$ [229]. U-shaped protective curve was achieved for galantamine (concentration 0.3 μM), for donepezil (1 μM concentration); rivastigmine showed a concentration-dependent effect with the maximum at 3 μM. The blocker of phosphoinositide 3-kinase (PI3K)-Akt reversed the protective effects of galantamine, donepezil, but not the effect of rivastigmine [229].This mechanism of action is likely not related to AChE inhibition [229]. Additive protective mechanism of donepezil was related to inhibition of glycogen synthase kinase 3 (GSK-3) and Akt activation [230]. Additionally, effects of donepezil against glutamate neurotoxicity were observed in primary cultures from rat brain cortex; incubation of cortical neurons prevented glutamate-induced apoptosis and consequently apoptotic neuronal death [231].

Donepezil was found to cause the loss of $\Delta\psi_m$, to increase the release of cyt *c* to the cytosol, and to alter the expressions of Bcl-2 family proteins [232]. Donepezil displayed an induction of apoptosis in HL-60 cells via a mitochondria-mediated caspase-dependent pathway [232]. Mitochondrial membrane depolarization was significantly visible at 24 h after treatment with donepezil and was further increased at 48 h. The study suggested that donepezil induced the activation of caspase-9 mediated by loss of $\Delta\psi_m$ in HL-60 cells. Loss of $\Delta\psi_m$ is usually associated

with the formation of mPTPs and diffusion of cyt *c*, which is normally associated with the inner mitochondrial membrane, into the cytosol. Cytosolic cyt *c* activates procaspase-9 by binding to apoptotic protease-activating factor-1 (Apaf-1), which leads to caspase-9 activation and the subsequent activations of downstream executioner caspases (caspases-3, -6 and -7) [232]. Levels of cytosolic cyt *c* were elevated by donepezil in HL-60 cells, which suggested the involvement of the mitochondrial pathway in donepezil-induced apoptosis. These findings suggest that donepezil modulates the protein levels of Bid, Bax, Bcl-2, and Bcl-xl, and that this results in loss of $\Delta\psi_m$ and release of cyt *c* from mitochondria [232].

Rivastigmine is a non-competitive pseudo-irreversible inhibitor [233, 234]. It differs from tacrine and donepezil in its structure and pharmacokinetic properties [234]; rivastigmine inhibits both AChE and BChE, with its long inhibition on AChE acting up to 10 hours. Rivastigmine was also reported to be responsible for decline of AChE activity in CSF of AD patients [235-237]. Biochemical studies showed that rivastigmine induces greater selectivity of AChE inhibition in the CNS than in the periphery [234, 238]. Therefore, AD patients, deteriorating on selective AChE inhibitor or are unable to tolerate treatment with donepezil, can benefit from a switch to rivastigmine [239, 240]. As a dual inhibitor, rivastigmine could provide more sustained efficacy than selective AChE inhibitors and help to slow the formation of amyloidogenic proteins [241].

In degenerating primary rat neurons rivastigmine decreased Aβ secretion and increased α-secretase cleaved secreted APP [242]. Elevated levels of α-secretase cleaved secreted APP could participate in cellular metabolic activity and enhanced neuronal survival [242].

Biochemical analysis performed by Kumar et al. [243] revealed the possible role of rivastigmine against 3-nitropropionic acid (complex II inhibitor) induced behavioural, biochemical and cellular alterations. Further, rivastigmine treatment significantly attenuated oxidative damage and improved mitochondrial complexes enzyme activities in different regions (striatum, cortex and hippocampus) of rat brain. The results show that rivastigmine could be used as an effective therapeutic agent in the management of several neurodegenerative including AD and Huntington's disease.

Rivastigmine exerts a profound effect on lymphocyte mitochondria [244]. A pattern of higher oxidative and enzymatic activities was seen in rivastigmine treated-AD patients when compared with control or untreated-AD. Statistically significant difference was observed for oxidation of pyruvate-malate (substrate of complex I) and glycerol-3-phosphate (substrate of complex III),

and for enzymatic activities of complexes II, III and IV. The differences were always present between treated-AD and untreated-AD patients and, in most cases, between treated-AD patients and controls. Rivastigmine enhanced the mitochondrial ability to oxidize substrates for complexes I and III (respiratory capacity), and increased the enzymatic activity of ETC complexes II, III and IV. Although without statistical significance, it stimulated physiological respiration and ability to oxidize succinate, a substrate for complex II. The mechanisms by which rivastigmine would stimulate ETC are uncertain.

Galantamine has dual mechanism; it acts as competitive reversible AChE inhibitor and allosterically potentiates nicotinic acetylcholine receptors [245]. Allosteric modulation of nicotinic acetylcholine receptors could have therapeutic benefit in AD [246]. Study using human neuroblastoma SH-SY5Y cells showed increased density of α7 nicotinic receptors and up-regulation of antiapoptotic protein Bcl-2 after the incubation with galantamine [247]. Another *in vitro* study observed that galantamine protected PC12 cells against the Aβ-induced apoptosis. It prevented Aβ aggregation, morphological changes of endoplasmic reticulum and mitochondria, and loss of $\Delta\psi_m$ as well as accumulation of ROS [248]. In human HePG cells galantamine did not induce cytotoxicity, it did not affect oxidative stress markers (increased malondialdehyde production, decreased in glutathione levels), and did not increased ROS production in concentrations lower than 100 μM [249]. Galantamine significantly restored complex I and complex II activity in mice, where the neurodegeneration was induced by intrahippocampal administration of kainic acid [250].

Memantine, non-cholinergic alternative to AChE inhibitors, a low affinity uncompetitive antagonist of NMDA receptors has been approved for AD treatment of moderate to severe stage of the disease. Memantine preferentially blocks extensive activity of NMDA receptors without disruption of normal physiological activity, it does not accumulate to interfere with normal synaptic transmission [251, 252]. Inhibition of NMDA receptors by memantine prevented the mitochondrial swelling, increased oxidative stress and decreased $\Delta\psi_m$ induced by berberine *in vitro* in primary neurons from mice and rats [253]. Memantine prevented *in vitro* irreversible electrophysiological changes induced by inhibitor of complex II, 3-nitropropionic acid, but it was not effective in protection of complex I, the rotenone toxicity (complex I inhibitor) was not influenced in spiny striatal neurons [254]. Similarly to ChE inhibitors, memantine reduced Aβ

levels in neuronal cultures in APP/PS1 transgenic mice [255]. Memantine attenuated the mitochondrial dysfunctions, reduced mitochondrial calcium, reversed $\Delta\psi_m$, and also reduced the ROS production (donepezil had no effect on ROS levels) induced by okadaic acid in rat brain mitochondria [228]. Arif et al. aimed to investigate the protective effects of memantine on $A\beta_{25-35}$ induced changes in peptidergic and glial systems [256]. Treatment with memantine appreciably increased $A\beta_{25-35}$ induced changes of neuropeptides, their metabolizing enzymes and glial marker proteins. They suggested that memantine exerts its protective effects by modulating the neuropeptide system as a consequence of suppressing the glial cells and oxidative stress in AD model rat brain. The memantine protection against Aβ induced neurotoxicity and learning impairment was reported in rats [257]. They concluded that memantine, at therapeutically relevant concentrations, can protect against neuronal degeneration induced by Aβ. The probable role of memantine against Aβ-induced toxicity; memantine treatment remarkably protected cultured neurons against Aβ-induced toxicity by attenuating tau-phosphorylation and its related signaling mechanisms [258]. However, this drug did not alter either conformation or internalization of $A\beta_{1-42}$ and it was unable to attenuate Aβ-induced potentiation of extracellular glutamate levels.

2.6 Novel drugs in (pre)clinical studies and their mechanisms of action

Huperzine A, an active alkaloid from Lycopodium, was described as a selective and reversible AChE and BChE inhibitor, with neuroprotective effects against the glutamate induced toxicity and anti-inflammatory properties [259, 260]. Interaction with excitatory amino acid neurotransmitter system and subsequent glutamate-induced calcium mobilization was observed after the incubation of neuronal cultures with huperzine A, reduction of NMDA mediated toxicity has been found. This blockage of NMDA ion channels without any psychomimetic effects could be useful in diverse neurodegenerative diseases [261]. Next to these effects, huperzine A has been described as disease modifying drug with neuroprotective effects directly acting on mitochondria [262]. It ameliorated the effect of $A\beta_{1-42}$ on ATP reduction and mitochondrial swelling, as well as a decrease in the enzymatic activities of respiratory chain complexes (complex II/III and complex IV) in isolated brain mitochondria from double transgenic AβPP/PS1 mice [263]. In APP/PS1 double-transgenic mice and SH-SY5Y cells huperzine A

inhibited GSK-3β and increased the level of β-catenin. These findings suggest that the neuroprotective effect of huperzine A can be related to the targeting of the Wnt/β-catenin signaling pathway [264].

Compared with tacrine, donepezil, and rivastigmine, huperzine A has better penetration through the blood-brain barrier, higher oral bioavailability, and longer duration of AChE inhibitory action and fewer peripheral cholinergic side effects [265]. Huperzine A possesses the ability to protect cells against H_2O_2, Aβ, glutamate, ischemia, staurosporine-induced cytotoxicity and apoptosis [265]. Donepezil, galantamine as well as huperzine A increased the viability of neurons against $A\beta_{42}$ toxicity [230]. On cellular level, donepezil reduced calcium-induced mitochondrial swelling in APP/PS1 transgenic mice [266].

Tacrine was the first registered reversible ChE inhibitor. It was withdrawn from the market due to its poor selectivity towards AChE, which resulted in a number of adverse effects (hepatotoxicity, gastrointestinal discomfort) [267]. Recently, novel tacrine and **7-methoxytacrine (7-MEOTA) derivatives** were synthesized and extensively investigated to find less toxic compounds affecting more AD pathological mechanisms. 7-MEOTA derivatives have lower toxicity while retaining pharmacological properties of tacrine and are promising candidates for AD treatment [268]. Their ability to inhibit AChE and BChE were evaluated on recombinant human AChE and plasmatic human BChE. An effort from our group [269] was to examine the effect of ChE inhibitors (tacrine, 7-MEOTA) on the activity of complex I in brain mitochondria. Inhibition of complex I by tacrine was statistically significant, which suggested the possibility of tacrine-induced side effects related to disturbances of ETC. Further *in vitro* testing is necessary for evaluation of new tacrine derivatives as candidate molecules for the treatment of AD.

Synthesized hybrid cholinesterase inhibitors

The multifactorial and complex nature of AD makes inadequate the use of magic bullets targeted to a single receptor or enzymatic system for the efficient treatment of the disease. However, it is now widely accepted that a more effective therapy would result from the use of multipotent compounds able to intervene in the different pathological events underlying the etiology of AD [270].

Tacrine-derived hybrid drugs

Additionally to tacrine and 7-MEOTA, tacrine-flavonoids, tacrine-coumarins, tacrine-trolox hybrids and tacrine-propargylamine derivatives have been recently designed, synthesized and evaluated as multifunctional ChE inhibitors [271-276]. Tacrine-propargyline derivatives exhibited balanced AChE and BChE activities, increased human AChE activity, whereas lower neurotoxicity and hepatotoxicity compared to tacrine [273].

Tacrine-coumarin hybrids exhibited an ability to inhibit ChE and induced self-Aβ-aggregation, they acted also as metal chelators [276]. A new series of tacrine-flavonoid hybrids were designed and synthesized, most of the compounds inhibited both AChE and BChE activities [272]. A tacrine-flavonoid hybrid was found to be a potent and balanced inhibitor against ChE, and induced self-aggregation with $A\beta_{1-42}$ [272]. Combining tacrine with trolox, a strong antioxidant, resulted in synthesis of molecule, which was less hepatotoxic than tacrine, strong inhibitor of AChE and BChE showing neuroprotective effects [275]. Another designed tacrine hybrid was presented with the ability to inhibit AChE and BChE, good inhibition of Aβ aggregation and good antioxidant activity [271]. Phenylthiazole-tacrine hybrids were examined as cholinesterase (ChE) inhibitors, blocking $A\beta_{1-42}$ aggregation and calcium overload [274].

Donepezil-derived hybrid drugs

Donepezil-indolyl hybrids were designed, synthesized and pharmacologically evaluated as multipotent ASS234 analogues (amines, amides, carboxylic acids), which are able to inhibit simultaneously ChE and MAO enzymes [277]. By performing *in vitro* analysis, it was concluded that the amines are in general more potent ChE inhibitors or equipotent than the corresponding amides. Amides were not active in inhibition of MAO; among the amines, several compounds were MAO-A selective. Two carboxylic acid derivatives showed a multipotent moderate selective profile as AChE and MAO-A inhibitors. As a result N-(5-(3-(1-benzylpiperidin-4-yl)propoxy)-1-methyl-1H-indol-2-yl)methyl)prop-2-yn-1-amine (15),was identified as a potent inhibitor of MAO-A (IC_{50}, 5.5±1.4 nM), moderately potent inhibitor of MAO-B (IC_{50}, 150±31 nM), and inhibitor of both AChE (IC_{50}, 190 ± 10 nM), and BChE (IC_{50}, 830±160 nM) [277].

Inhibitors of MAO-B and derived hybrid drugs

Propargyline derivatives, selegiline and rasagiline, are selective irreversible MAO-B inhibitors used in PD treatment. High expression levels of MAO-B can result in increased level of ROS and might play a role in etiology of AD.

Selegiline has antioxidant and neuroprotective effects; it reduced lipid peroxidation in prefrontal cortex, striatum and hippocampus and increased the activity of glutathione dismutase activity; these effects can attenuate the neurodegenerative processes in aged rats [278, 279]. New carbamate derivatives of aminoindans (rasagiline-related series) and phenylethylamines (selegiline-related series) have been described as dual inhibitors of AChE and MAO [280]. Novel compounds were designed using a combination of benzylpiperidine moiety of donepezil and indolylpropargylamino moiety of MAO inhibitor [281].

A series of tacrine-selegiline hybrids was synthesised and evaluated as inhibitors of cholinesterase (AChE/BChE) and MAO-A/B [282]. The results demonstrated that most of the synthesised compounds exhibit high inhibitory activity. Among these compounds, compound **8g** provided a good balance of activity towards all targeted enzymes: AChE, BChE, MAO-A and MAO-B, respectively. These results indicated that **8g** has the potential to be a multi-functional candidate for AD.

The novel drug ladostigil tartrate (TV-3326) is derived from a combination of two pharmacophores: the carbamate moiety from rivastigmine, an AChE inhibitor, and propargyl group from rasagiline, a MAO-B inhibitor. This drug exhibits both ChE and selective MAO inhibitory activities in the brain, reduces apoptosis and stimulates the processing of APP, hence reducing the possibility of generating the toxic Aβ [283].

A series of novel propargylaminoindans with a carbamate moiety to inhibit ChE were developed from pharmacophore of rasagiline to protect or rescue deteriorated neurons in AD and Lewy body disease and provide a beneficial effect on the cognitive deficits [284]. The carbamate moiety of rivastigmine was introduced into the 6 position of the rasagiline molecule to provide ChE inhibitory activity [285-287]. Rasagiline analogues were found to protect dopaminergic SH-SY5Y cells against apoptosis induced by peroxynitrite donor, SIN-1. TV3326, [(N-propargyl)-(3R)-aminoindan-5-yl]-ethyl methyl carbamate, was as effective as rasagiline in preventing

apoptosis, followed by its S-enantiomer, TV3279. The anti-apoptotic-neuroprotective activity was shown to reside in the propargylamine and not the carbamate moiety. This resulted in stabilization of the $\Delta\psi_m$, the collapse of which initiates the apoptotic cascade.

Neuroprotective activities of TV3326, its S isomer, TV3279, and related compounds were examined for their potential protection against apoptosis and the fall in $\Delta\psi_m$ associated with apoptosis induced by the peroxynitrite-generating agent, SIN-1 [N-morpholinosydnonimine] in dopaminergic neuroblastoma SH-SY5Y cells [284]. The presence of the carbamate moiety in TV3326 and TV3279, did not affect the anti-apoptotic function associated with rasagiline. The metabolite TV3218, devoid of propargyl moiety, was devoid of anti-apoptotic activity. By contrast the hydroxylpropargyl major metabolite of TV3326, TV3294, had anti apoptotic activity similar to rasagiline and TV3326 and TV3279.

The neuroprotective effects of tacrine hybrids could be associated with the inhibition of AChE induced Aβ aggregation and inhibition of β-secretase. Two isomeric series of dual binding site AChE inhibitors have been designed and synthesized by Camps et al. [288]. These hybrids, consisting of a unit of 6-chlorotacrine and pyrano[3,2-c]quinoline, possessed the potent and selective human AChE inhibitory activity and exhibited a significant *in vitro* inhibitory activity toward the AChE-induced and self-induced Aβ aggregation and toward β-secretase, in addition to the ability to enter the central nervous system [289].

Donepezil + propargylamine + 8-hydroxyquinoline hybrids (DPH) are multifunctional metal-chelators, ChE and MAO inhibitors. They have been newly synthesized and biochemically evaluated for potential AD treatment investigated [290].The most interesting derivative was racemic derivative α-aminotrile-4-(1-benzylpiperidin-4-yl)-2-(8-hydroxyquinolin-5-yl)methyl)(prop-2-yn-1-yl)amino)butanenitrile (DPH6). It was characterized as irreversible MAO-A/B inhibitor and mixed-type AChE inhibitor with metal-chelating properties [MAO-A (IC_{50} = 6.2±0.7 μM; MAO-B (IC_{50} = 10.2 ± 0.9 μM); AChE (IC_{50} = 1.8 ±0.1 μM); BChE (IC_{50} = 1.6 ± 0.25 μM)]. These DPH hybrids performed good blood brain barrier penetration and were found to be less toxic in an *in vitro* tested toxicity in HepG2 cells [290].

Liu et al. [291] have investigated the effects of N-[2-(4-hydroxyphenyl)ethyl]-2-(2,5-dimethoxyphenyl)-3-(3-methoxy-4-hydroxyphenyl)acrylamide, compound FLZ, a novel synthetic analogue of squamosamide, on the dysfunction of rat brain mitochondria induced by $A\beta_{25–35}$ *in vitro*. The activities of mitochondrial enzymes, production of H_2O_2 and superoxide anion and the levels of glutathione in mitochondria were examined. Incubation of mitochondria with aged $A\beta_{25–35}$ inhibited the activities of α-ketoglutarate dehydrogenase, pyruvate dehydrogenase and complex IV of electron transport chain (ETC). Increased H_2O_2 and superoxide anion production, and decreased the glutathione level were observed. Furthermore, it induced mitochondrial swelling and cyt *c* release from the mitochondria. The addition of FLZ (100 μmol/L) prior to treatment with $A\beta_{25–35}$ significantly prevented these toxic effects of $A\beta_{25–35}$ on rat brain mitochondria and *in vitro* protective effects of FLZ were concluded. Another study examined the effects of FLZ in APP-SH-SY5Y cells, FLZ selectively inhibited γ-secretase and decreased accumulation of Aβ in mitochondria [292]. The inhibitory effect of FLZ in APP/PS1 double transgenic mice and SH-SY5Y cells on GSK-3β activity and tau phosphorylation was observed. FLZ inhibited also Akt activity, which indicated that Akt/GSK-3β pathway might be the possible mechanism of involved in the inhibitory effect of FLZ on tau hyperphosphorylation. These results suggested FLZ as potential for AD therapy, reducing Aβ production via both mechanisms inhibition amyloidogenic APP processing pathway and attenuated tau hyperphosphorylation mediated by Akt/GSK-3β [293].

2.7 Drugs interacting with mitochondrial enzymes

Aβ forms extracellular plaques, in the cell Aβ interacts with intracellular targets such as mitochondrial proteins, amyloid-beta binding alcohol dehydrogenase (ABAD, also 17-β-hydroxysteroid dehydrogenase X, HSD10) modulators and cyclophilin D (CypD) [294]. ABAD is dehydrogenase interacting with Aβ and promoting Aβ-mediated mitochondrial and neuronal dysfunctions [295]. Aβ-ABAD complex was examined by high-resolution crystallography, deformation of ABAD structure with exclusion of NAD^+ cofactor was demonstrated in the Aβ presence [296]. ABAD enhanced Aβ-induced cell stress via decreased COX activity and exacerbated leakage of ROS in neurons, these changes were found in cultured neurons from transgenic mAPP/ABAD mice. Therefore, design of **ABAD modulators** and targeting of

mitochondrial ABAD represent a novel strategy of AD treatment; synthesized ABAD modulators (ABAD-4a, ABAD-4b) increased the COX activity and ATP levels and suggested the protective effects on mitochondrial properties [295]. Frentizole, immunosuppressive drug, was identified as inhibitor of Aβ-ABAD interaction; other benzothiazole urea derivatives and frentizole analogues have been developed [298].

The role of CypD-dependent mPTP was reported in Aβ-impaired axonal mitochondrial trafficking [299]. Depletion of CypD protects axonal mitochondrial motility and dynamics from Aβ toxicity as revealed by augmented axonal mitochondrial density and distribution and improved bidirectional transport of axonal mitochondria. Notably, blockade of mPTP by genetic deletion of CypD suppresses Aβ-mediated activation of the p38 mitogen-activated protein kinase signaling pathway, reverses axonal mitochondrial abnormalities, recovers synaptic function, and reduces loss of synapse, suggesting a role of CypD-dependent signaling in Aβ-induced alterations in axonal mitochondrial trafficking. Very interesting review summarizing the progress on mPTP and its potential therapeutic target for neurodegenerative diseases including AD was presented by Rao [299]. The authors have also reported that interaction of CypD with mitochondrial Aβ potentiates mitochondrial, neuronal and synaptic stress [300]. Their findings have manifested that the CypD and Aβ directly interact with each other in the mitochondria of AD brain and in a transgenic mouse model of AD. According to their study, CypD-Aβ interaction promotes ROS generation and CypD recruitment to the mitochondrial inner membrane, leading to the formation of the mPTP. The CypD-deficient cortical mitochondria are resilient to Aβ- and Ca^{2+}-induced mitochondrial swelling and permeability transition. They have better calcium buffering capacity and generate fewer mitochondrial ROS. The absence of CypD protects neurons from Aβ- and oxidative stress-induced cell death. Notably, CypD deficiency significantly develops learning and memory and synaptic function in an AD mouse model and improves Aβ-mediated reduction of long-term potentiation. Thus, the CypD-mediated mitochondrial permeability transition pore is directly linked to the cellular and synaptic perturbations observed in the pathogenesis of AD.

Novel series of quinazoline-urea derivatives as modulators of Aβ induced mitochondrial dysfunctions were prepared and studied [301]. Their blocking activities against Aβ induced disruption of $\Delta\psi_m$ and led to mitochondrial mPTP. From the results the active nonpeptidyl **mPTP blockers** can be considered as a new direction for the design of novel mPTP modulators.

Summary to published papers

3. *In vitro* models

3.1 Pig brain mitochondria as a biological model for study of mitochondrial respiration

Fišar Z, Hroudová J: Pig brain mitochondria as biological model for study of mitochondrial respiration. Folia Biol (Praha). 2016; 62(1):15-25. (IF 2015 = 0.833)

Isolated mitochondria serve as a biological model for understanding the mitochondrial respiration control, effects of various biologically active substances, and pathophysiology of mitochondrial diseases. The aim of our study was to evaluate pig brain mitochondria as a proper biological model for investigation of activity of the mitochondrial ETC. Oxygen consumption rates of isolated pig brain mitochondria were measured using high-resolution respirometry. Mitochondrial respiration of crude mitochondrial fraction, mitochondria purified in sucrose gradient, and mitochondria purified in Percoll gradient were assayed as a function of storage time. Oxygen flux and various mitochondrial respiratory control ratios were not changed within two days of mitochondria storage on ice. LEAK respiration (state 4, non-phosphorylating resting state) was found higher and Complex I-linked respiration lower in purified mitochondria compared to the crude mitochondrial fraction. Damage to both outer and inner mitochondrial membrane caused by the isolation procedure was the greatest after purification in a sucrose gradient. We confirmed that pig brain mitochondria can serve as a biological model for investigation of mitochondrial respiration. The advantage of this biological model is the stability of respiratory parameters for more than 48 h and the possibility to isolate large amounts of mitochondria from specific brain areas without the need to kill laboratory animals. We suggest the use of high-resolution respirometry of pig brain mitochondria for research of the neuroprotective effects and/or mitochondrial toxicity of new medicinal drugs.

3.2 Mitochondrial DNA mutations and oxidative phosphorylation (cybrid cells)

3.2.1 Capture of somatic mtDNA point mutations with effects on OXPHOS in cybrids

McKenzie M, Chiotis M, Hroudová J, Lopez Sanchez MI, Lim SC, Cook MJ, McKelvie P, Cotton RG, Murphy M, St John JC, Trounce IA: Capture of somatic mtDNA point mutations

with severe effects on oxidative phosphorylation in synaptosome cybrid clones from human brain. Human Mutation 2014; 35(12): 1476-1484 (IF 2014 = 5.340)

MtDNA is replicated throughout life in postmitotic cells, resulting in higher levels of somatic mutation than in nuclear genes. However, controversy remains as to the importance of low-level mtDNA somatic mutants in cancerous and normal human tissues. To capture somatic mtDNA mutations for functional analysis, we generated synaptosome cybrids from synaptic endings isolated from fresh hippocampus and cortex brain biopsies. We analyzed the whole mtDNA genome from 120 cybrid clones derived from four individual donors by chemical cleavage of mismatch and Sanger sequencing, scanning around two million base pairs. Seventeen different somatic point mutations were identified, including eight coding region mutations, four of which result in frameshifts. Examination of one cybrid clone with a novel m.2949_2953delCTATT mutation in MT-RNR2 (which encodes mitochondrial 16S rRNA) revealed a severe disruption of mtDNA-encoded protein translation. We also performed functional studies on a homoplasmic nonsense mutation in MT-ND1, previously reported in oncocytomas, and show that both ATP generation and the stability of OXPHOS complex I are disrupted. As the mtDNA remains locked against direct genetic manipulation, we demonstrate that the synaptosome cybrid approach can capture biologically relevant mtDNA mutants *in vitro* to study effects on mitochondrial respiratory chain function.

3.2.2 Loss of mtDNA-encoded protein ND1 results in disruption of complex I biogenesis

Lim SC, Hroudová J, Van Bergen NJ, Lopez Sanchez MI, Trounce IA, McKenzie M. Loss of mitochondrial DNA-encoded protein ND1 results in disruption of complex I biogenesis during early stages of assembly. FASEB Journal 2016; 30(6): 2236-48. (IF 2015 = 5.299)

Mitochondrial complex I must be assembled precisely from 45 protein subunits for it to function correctly. One of its mitochondrial DNA (mtDNA) encoded subunits, ND1, is incorporated during the early stages of complex I assembly. However, little is known about how mutations in ND1 affect this assembly process. We found that in human 143B cybrid cells carrying a homoplasmic MT-ND1 mutation, ND1 protein could not be translated. As a result, the early stages of complex I assembly were disrupted, with mature complex I undetectable and

complex I-linked respiration severely reduced to 2.0% of control levels. Interestingly, complex IV steady-state levels were also reduced to 40.3%, possibly due to its diminished stability in the absence of respiratory supercomplex formation. This was in comparison with 143B cybrid controls (that contained wild-type mtDNA on the same nuclear background), which exhibited normal complex I, COX, and supercomplex assembly. We conclude that the loss of ND1 stalls complex I assembly during the early stages of its biogenesis, which not only results in the loss of mature complex I but also disrupts the stability of complex IV and the respiratory supercomplex to cause mitochondrial dysfunction.

4 *In vitro* effects of drugs

4.1. Effects of antidepressants and mood stabilizers on mitochondrial respiration

Hroudová J, Fišar Z: *In vitro* inhibition of mitochondrial respiratory rate by antidepressants. Toxicology Letters 2012; 213(3): 345-352 (IF 2012 = 3.145)

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)

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 4 and 5.

Table 4 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 5 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.

Among the tested antidepressants, fluoxetine and tianeptine were the most potent inhibitors of respiration supported by substrates for electron supply through complex I. Tianeptine, but not fluoxetine was potent inhibitor of respiration supported by substrates for electron supply through complex II. Ketamine 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 4 and 5); it indicates that only partial inhibition of respiratory rate occurs.

In vitro effects of pharmacologically different antidepressants (desipramine, amitriptyline, imipramine, citalopram, venlafaxine, mirtazapine, tianeptine, and moclobemide) and three mood stabilizers (lithium, valproate, and olanzapine) on the activities of mitochondrial enzymes (CS and enzymes in ETC, i.e. complexes I, II, IV) were measured in crude mitochondrial fraction isolated from pig brain.

Activities of ETC complexes were mostly decreased owing to tested antidepressants and mood stabilizers; the most affected was complex I and IV (Fig. 1).

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. Considering very high final drug concentrations in samples the values showed on Fig. 1 conform to residual activities of enzyme complexes.

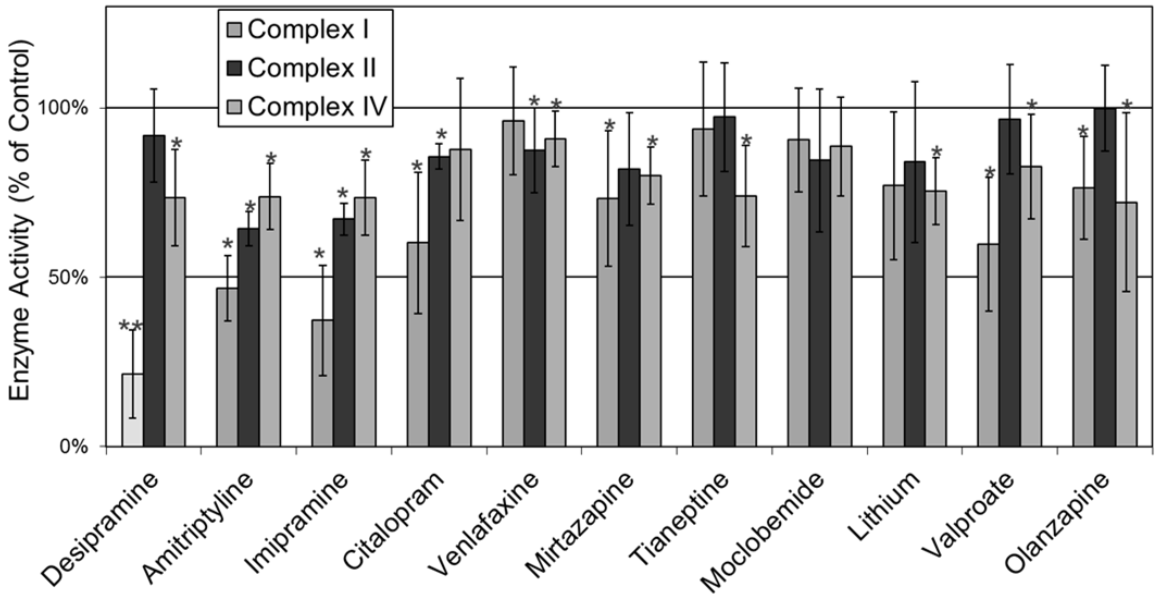


Fig. 1 Effects of antidepressants and mood stabilizers on activities of the respiratory chain complexes I, II, IV in a brain crude mitochondrial fraction. Relative activities are displayed (100 % = control sample without the drug). Values are means ± standard deviation of at least five independent measurements. Comparisons between controls and samples with drug were performed using the Wilcoxon matched pairs test (*$p<0.05$).

Potency of tested drug in affecting of citrate synthase (CS) activity is summarized in the Table 6. Except for mirtazapine and moclobemide all tested drugs slightly increased CS activity; however, the increase was statistically significant only for citalopram, tianeptine and olanzapine. Our results indicate that antidepressants may act generally as inhibitors of complex I and complex IV of the ETC. These mitochondrial enzymes are suggested as proper candidates in searching of new biological markers of mood disorders, targets of new antidepressants or predictors of response to pharmacotherapy.

Table 6 Effects of antidepressants and mood stabilizers on 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	-	-

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; CS – citrate synthase, SDH - succinate dehydrogenase.

4.2 Effects of cognitives and nootropics on mitochondrial respiration

Singh N, Hroudová J, Fišar Z. *In vitro* effects of cognitives and nootropics on mitochondrial respiration and monoamine oxidase activity. Molecular Neurobiology 2017; 54: 5894-5904 (IF 2016 = 6.190)

Effects of drugs used for symptomatic AD treatment may be related to their direct action on mitochondrial functions. *In vitro* effects of pharmacologically different cognitives (galantamine, donepezil, rivastigmine, 7-MEOTA, memantine), and nootropic drugs (latrepirdine, piracetam) were investigated on selected mitochondrial parameters: activities of ETC complexes I, II + III, and IV, CS, MAO, oxygen consumption rate, and H_2O_2 production of pig brain mitochondria. Complex I activity was decreased by galantamine, donepezil, and memantine; complex II + III activity was increased by galantamine. None of the tested drugs caused significant changes in the

rate of mitochondrial oxygen consumption, even at high concentrations. Except galantamine, all tested drugs were selective MAO-A inhibitors.

Latrepirdine, donepezil, and 7-MEOTA were found to be the most potent MAO-A inhibitors. Succinate-induced mitochondrial H_2O_2 production was not significantly affected by the drugs tested. The direct effect of cognitives and nootropics used in the treatment of AD on mitochondrial respiration is relatively small. The safest drugs in terms of disturbing mitochondrial function appear to be piracetam and rivastigmine. The MAO-A inhibition by cognitives and nootropics may also participate in mitochondrial neuroprotection. The results support the future research aimed at measuring the effects of currently used drugs or newly synthesized drugs on mitochondrial functioning in order to understand their mechanism of action.

4.3 *In vitro* evaluation of new tacrine derivatives, AChE inhibitors and oximes

Korabecny J, Janovec L, Musilek K, Zemek F, Horova A, Nepovimova E, Dolezal R, Opletalova V, Hroudova J, Fisar Z, Jung Y, Kuca K: Comparison of novel tacrine and 7-MEOTA derivatives with aromatic and alicyclic residues: synthesis, biological evaluation and docking studies. Letters in Organic Chemistry 2013; 10(4): 291-297 (IF 2013 = 0.648)

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 2011 = 2.554)

Korábečný J, Musílek K, Holas O, Zemek F, Opletalová V, Dohnal V, Nachon F, Hroudová J, Fišar Z, Kuča 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 concern 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 2011 = 1.296)

New analogs of tacrine and 7-MEOTA were designed, synthesized, and biologically evaluated. Their inhibitory activities against both AChE and BChE were tested. Cholinergic properties were investigated and quantified with respect to their side chain residues (aromatic or alicyclic). All synthesized compounds proved to have potent inhibitory activity at micromolar range. One compound demonstrated promising efficacy and appears to be an ideal candidate for further testing [302].

A new tacrine based cholinesterase inhibitor, N-(bromobut-3-en-2-yl)-7-methoxy-1,2,3,4-tetrahydroacridin-9-amine, was designed and synthesized to interact with specific regions of human AChE and human BChE. Its inhibitory ability towards ChEs was determined and compared to tacrine and 7-MEOTA. This compound was found to be poor inhibitor of ChEs *in vitro*, but it showed 15-fold higher selectivity towards human AChE compared to tacrine [303]. Further, 7-MEOTA homodimers as by-products during tacrine-related synthesis have been developed. As resulted, newly prepared by-product showed cholinergic properties on both human AChE and human BChE. It is also probable that this compound will have lower toxicity if compared to with its tacrine analogue because it is derived from low toxic anticholinergic drug 7-MEOTA. This by-product was found as promising anti-AD agent and should be further tested [304].

Inhibition of the AChE is the main mechanism both of therapeutic action of drugs for the treatment of AD and toxic action of organophosphorus compounds. Various types of oximes reactivate AChE and are commonly used as antidotes against organophosphates (pesticides, nerve agents).

Effects of both AChE inhibitors (tacrine, 7-MEOTA) and oximes (pralidoxime, trimedoxime, obidoxime, methoxime, HI-6) on complex I of ETC were examined. The enzyme activity was measured spectrophotometrically in crude mitochondrial fraction isolated from pig brain. All drugs showed inhibitory effect on the complex I activity; however, only tacrine induced statistically significant inhibition.

Present results indicate that AChE reactivators pralidoxime, obidoxime, trimedoxime, methoxime and HI-6 can be most probably taken as relatively safe compound regarding to drug-induced changes in complex I activity and related changes in cellular energetics. It corresponds to study that observed influence of oximes on mitochondrial COX activity and showed only slightly

inhibited activity by 2-pyridinealdoxime methiodide (2-PAM) [305]. Contrary, tacrine significantly affect the complex I activity and we suppose that this effect can contribute to its adverse effects.

4.4 *In vitro* effects of drugs on MAO-A and MAO-B activities

4.4.1 Effects of antidepressants and mood stabilizers

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)

In general, the effect of drugs tested was found to be inhibitory. The half maximal inhibitory concentration, parameters of enzyme kinetic, and mechanism of inhibition were determined. MAO-A was inhibited by the following drugs: pargyline > clorgyline > iproniazid > fluoxetine > desipramine > amitriptyline > imipramine > citalopram > venlafaxine > olanzapine > reboxetine > mirtazapine > tianeptine > moclobemide, cocaine >> lithium, valproate. MAO-B was inhibited by the following drugs: pargyline > clorgyline > iproniazid > fluoxetine > venlafaxine > amitriptyline > olanzapine > citalopram > desipramine > reboxetine > imipramine > tianeptine > mirtazapine, cocaine >> moclobemide, lithium, valproate. The mechanism of inhibition of MAOs by several antidepressants was found various.

It was concluded that MAO activity is acutely affected by pharmacologically selective antidepressants at relatively high drug concentrations; this effect is inhibitory. There are differences both in inhibitory potency and in mechanism of inhibition both between several drugs and the two MAO isoforms. While MAO inhibition is not primary biochemical effect related to their therapeutic action, it can be supposed that decrease of MAO activity may be concerned in some effects of these drugs on serotonergic, noradrenergic, and dopaminergic neurotransmission.

4.4.2 Effects of pharmacologically different cognitives and nootropic drugs

Singh N, Hroudová J, Fišar Z. *In vitro* effects of cognitives and nootropics on mitochondrial respiration and monoamine oxidase activity. Molecular Neurobiology 2017; 54: 5894-5904 (IF 2016 = 6.190)

The dose-dependent effects of pharmacologically different cognitives and nootropic drugs on MAO-A and MAO-B activity were measured in pig brain mitochondria. MAO-A activity was fully inhibited by all tested drugs; however, higher half maximal inhibitory concentrations (IC_{50}) of cognitive enhancers were observed compared tothe known MAO inhibitors, such as iproniazid, pargyline, and clorgyline (Table 7). MAO-A was inhibited by the drugs tested in the following order: pargyline > clorgyline > iproniazid > donepezil = latrepirdine = 7-MEOTA > memantine = rivastigmine > galantamine > piracetam. Piracetam, galantamine, rivastigmine, and memantine are very weak inhibitors of MAO-A activity. Donepezil, latrepirdine, and 7-MEOTA are relatively strong MAO inhibitors. MAO-B activity was fully inhibited by latrepirdine and memantine; partial inhibition was caused by 7-MEOTA, donepezil, and galantamine. Rivastigmine induced an initial increase in MAO-B activity followed by a rapid decrease (full inhibition) at very high concentrations. Piracetam caused monotonic increase of MAO-B activity, even at very high (millimolar) concentrations. IC_{50} values characterizing MAO-B inhibition were higher than MAO-A inhibition, except for galantamine (Table 7).

Table 7 Inhibition of monoamine oxidase activity by cognitives and nootropics.

Drug	*MAO-A*		*MAO-B*		*MAO-A/MAO-B*
	IC_{50} (μmol/L)	*Inhibition*	*IC_{50} (μmol/L)*	*Inhibition*	
Piracetam	7668 ± 754	Full	increase	nd	
Latrepirdine	5.16 ± 0.39	Full	63.3 ± 8.9	Full	0.081
7-MEOTA	6.71 ± 0.72	Full	178 ± 13	Partial	0.038
Tacrine	9.84 ± 1.86	Full	502 ± 44	Full	0.020
Galantamine	1080 ± 137	Full	592 ± 564	Partial	1.826
Donepezil	5.04 ± 0.41	Full	105 ± 17	Partial	0.048
Rivastigmine	370 ± 41	Full	10085 ± 1034	(Full)	0.037
Memantine	313 ± 22	Full	4261 ± 666	Full	0.073

Drug	*MAO-A*		*MAO-B*		*MAO-A/MAO-B*
	IC_{50} (μmol/L)	*Inhibition*	*IC_{50} (μmol/L)*	*Inhibition*	
Pargyline	0.0116 ± 0.0007	Full	0.00853 ± 0.00039	Full	1.361
Clorgyline	0.326 ± 0.039	Full	0.224 ± 0.012	Full	1.454
Iproniazid	1.294 ± 0.098	Full	1.272 ± 0.071	Full	1.017

4.4.3 Effects of acetylcholinesterase reactivators

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 2011 = 3.230)

Administration of AChE reactivators (oximes) is usually used in order to counteract the poisoning effects of nerve agents. The possibility was suggested that oximes may show some therapeutic and/or adverse effects through their action in central nervous system. There are no sufficient data about interaction of oximes with monoaminergic neurotransmitter's systems in the brain. Oxime-type AChE reactivators pralidoxime, obidoxime, trimedoxime, methoxime and HI-6 were tested for their potential to affect the activity of MAO-A and MAO-B in crude mitochondrial fraction of pig brains. The compounds were found to inhibit fully MAO-A with half maximal inhibitory concentration (IC_{50}) of 0.375 mmol/l (pralidoxime), 1.53 mmol/l (HI-6), 2.31 mmol/l (methoxime), 2.42 mmol/l (obidoxime) and 4.98 mmol/l (trimedoxime). Activity of MAO-B was fully inhibited by HI-6 and pralidoxime only with IC_{50} 4.81 mmol/l and 11.01 mmol/l, respectively. Methoxime, obidoxime and trimedoxime displayed non-monotonic concentration dependent effect on MAO-B activity. Because oximes concentrations effective for MAO inhibition could not be achieved in vivo at the cerebral level, we suppose that oximes investigated do not interfere with brain MAO at therapeutically relevant concentrations.

The *in vitro* experiments suggest that oximes act as weak MAO-A inhibitors. Inhibitory parameters were calculated from total concentrations of oximes added to crude brain mitochondrial fraction. *In vivo* situation in tissues may be different from our experimental

conditions. However, at the same experimental conditions oximes exert much lower inhibitory potency than irreversible MAO inhibitor iproniazid (IC_{50} equals to 1.55 μmol/l for MAO-A, and 1.21 μmol/l for MAO-B) and lower or similar inhibitory potency than some antidepressants [112], antipsychotics [306] and endocannabinoids [307]. In conclusion, AChE reactivators pralidoxime, obidoxime, trimedoxime, methoxime and HI-6 have additional direct effects on MAO that are not related to AChE reactivation. The monoaminergic effects of oximes include weak MAO-A inhibition and very weak inhibitory or biphasic effects on MAO-B activity. Although it is always difficult to extrapolate from *in vitro* studies to the clinical reality, the present results suggest that the examined oximes might contribute to an inhibitory effect on MAO, and a systematic *in vivo* investigation of the oxime effect on MAO activity is needed. The effects of oximes on MAO activity may contribute to their nonreceptor actions participating in modulation of monoamine neurotransmission in the brain. However, the exact physiological role of MAO inhibition by oximes is not known. Nevertheless, present results indicate that AChE reactivators pralidoxime, obidoxime, trimedoxime, methoxime and HI-6 can be taken probably as the relatively safest compounds regarding to drug-induced changes in MAO activity and related to changes in monoaminergic neurotransmission.

5. Mitochondrial respiration in neuropsychiatric diseases

5.1 Mitochondrial respiration in blood platelets of depressive patients

Hroudová J, Fišar Z, Kitzlerová E, Zvěřová M, Raboch J: Mitochondrial respiration in blood platelets of depressive patients. Mitochondrion, 2013; 13(6): 795-800 (IF 2013 = 3.524)

A total of 21 depressive patients were recruited for the present study and performed clinical evaluation and measurements of mitochondrial respiration in blood platelets. The severity of depression was evaluated according to Hamilton rating scale for depression (HRSD). They were marked as "Depressive Patients (HRSD>10)" in the Table 8 and were characterized by HRSD>10 and CGI-S≥3. Of these, 12 were willing to perform follow-up measurements when partial remission was reached after some weeks of treatment (22.8 ± 9.5 days); they were marked as "Depressive Patients (HRSD≤10)" and were characterized by HRSD≤10, CGI-S≤3 and CGI-I≤2. Sixteen from twenty one depressive patients were treated primarily by SSRIs (escitalopram, sertraline, paroxetine, fluoxetine); however, antidepressants of other classes were also used (mirtazapine, venlafaxine, trazodone, agomelatine). Combined pharmacotherapy included often benzodiazepines; mood stabilizers and antipsychotics were used exceptionally (in four patients). The control group was recruited from 22 healthy volunteers; they were comparable with depressive subjects with regard to the age. Baseline characteristics of the depressive and control persons are shown in the Table 8. Data were evaluated together for women and men, because we did not observe significant difference between women and men in any measured variable.

Table 8 Baseline characteristics of participants

Subjects	*Age*	*HRSD-21*	*CGI-S*	*CGI-I*	*N*
Depressive patients (HRSD>10)	47.0 ± 14.2	20.9 ± 8.7	4.4 ± 1.0		21
Depressive patients (HRSD≤10)	43.4 ± 13.6	3.2 ± 2.2	2.2 ± 0.7	1.7 ± 0.4	12
Controls	44.7 ± 14.4	-	-	-	22

Depressive Patients (HRSD>10) = depressive patients with actual depressive episode characterized by HRSD > 10 and CGI-S ≥ 3; Depressive Patients (HRSD≤10) = depressive patients in partial remission characterized by HRSD ≤ 10, CGI-S ≤ 3 and CGI-I ≤ 2; HRSD = 21-item Hamilton Rating Scale for Depression; CGI-S = Clinical Global Impression - Severity scale; CGI-I = Clinical Global Impression - Improvement scale; N = number of participants

Mitochondrial respiration in intact platelets

In intact platelets, physiological respiration or maximal *p*-(trifluoromethoxy)phenylhydrazone (FCCP)-titrated capacity of ETS was significantly decreased in the "Depressive Patients (HRSD≤10)" group, when compared to healthy controls. The significance was sustained when respiration was related to the activity of CS. Significant increase was found in nonphosphorylating LEAK respiration in the "Depressive Patients (HRSD>10)" group. Respiratory rate after complex I inhibition was significantly lower in the "Depressive Patients (HRSD≤10)" group compared to both controls and "Depressive Patients (HRSD>10)" group.
Flux control ratios were calculated as ratios of respiratory rates in different respiratory states (Physiological Respiration - PR, LEAK, Net Physiological Respiration = Physiological Respiration - LEAK, Complex I Inhibition, and ROX) to maximal capacity of the electron transfer system (ETSC). Significant increase of LEAK/ETSC was found in patients with depressive symptoms. Efficiency of respiration after complex I inhibition was decreased in "Depressive Patients (HRSD≤10)" compared to controls.

Mitochondrial respiration in permeabilized platelets

In permeabilized platelets, mitochondria in different respiratory states were studied. The State 3 CI respiration was induced by addition of malate, pyruvate, adenosine 5´-diphosphate (ADP) and glutamate, the State 3 CI+II by addition of succinate. The LEAK state was measured as oligomycin induced state 4. State ETSC reflecting the maximal capacity of the electron transfer system was determined by titration with FCCP, and Complex I Inhibition was determined by addition of rotenone. We did not observe significant change either in respiratory rate or in flux control ratio in any respiratory state; the same results were obtained when respiration was related to the CS activity.

5.2 Mitochondrial respiration in blood platelets of BD patients

Hroudová J, Fišar Z, Kališová L, Kitzlerová E, Zvěřová M, Sigitova E, Hansíková H, Raboch J: Mitochondrial dysfunctions in blood platelets of patients with manic episode of bipolar disorder. Progress in Neuro-Psychopharmacology & Biological Psychiatry, submitted (IF 2016 = 4.187)

17 patients hospitalized for acute manic episode of BD were recruited for the present study and underwent clinical evaluation and mitochondrial function measurements in blood platelets. Clinical data include scales: Young Mania Rating Scale (YMRS); brief psychiatric rating scale (BPRS); scores of the scales are summarized in Table 9. Mood disorder questionnaire (MDQ) was used to confirm diagnosis of BD, all patients met the criteria for BD.

Table 9 Baseline characteristics of participants

Subjects	*Age*	*YMRS*	*CGI-01*	*BPRS*	*N*
BAD patients in mania	39.4 ±12.8	24.3 ± 11.3	5.5 ± 0.9	63.9 ± 16.6	17
Controls	41.1 ± 12.5	-	-	-	25

Young Mania Rating Scale (YMRS); clinical global impression - (CGI - severity of illness); Brief psychiatric rating scale - 24 items version (BPRS); N = number of participants

BPRS: The BPRS evaluates the overall psychiatric status, it includes 24-items evaluating severity of psychopathology symptoms on 7 - point Likert scale. Overall Scores for this scale range from 24-168 (higher scores indicating more psychopathology) [308].

CGI 1: The CGI indicates the severity of illness. 1- normal, 2- borderline mentally ill, 3 – mildly ill, 4 moderately ill, 5- markedly ill, 6 severely ill a 7 among most extremely ill patients [309].

YMRS: result corresponds to the severity of the disorder, e.g. YMRS score higher than 25 corresponded to CGI higher than 5 (markedly ill) [310].

CS activity was not changed in BD patients compared to controls. Ratio complex I to CS was found significantly increased.

Mitochondrial respiration in intact platelets

In intact platelets, *PR* ($p = 0.002$) and *LEAK* ($p = 0.007$) were significantly increased, and *Rot* ($p = 0.000$x) was decreased in BD patients compared to controls. ETSC was not significantly changed. When the mitochondrial respiration was normalized to CS activity, the ratios PR/CS ($p = 0.000$) and LEAK/CS ($p = 0.006$) were significantly increased and the ratios Rot/CS ($p = 0.000$) and ROX/CS ($p = 0.000$) were significantly decreased in platelets from BD patients with

manic episode compared to controls. When the mitochondrial respiration was normalized to ETS capacity, which represents an internal functional mitochondrial marker, ratios PR/ETSC (p = 0.000), LEAK/ETSC (p = 0.007) and NetPR/ETSC (p = 0.000) were significantly increased and Rot/ETSC (p = 0.000) was significantly decreased in blood platelets from BD patients with manic episode.

Mitochondrial respiration in permeabilized platelets

In permeabilized platelets from BD patients, ETSC was significantly decreased (p = 0.004). When the respiratory rates were normalized to CS activity, ETSC/CS was found to be also significantly decreased (p = 0.04).

5.3 Mitochondrial respiration in blood platelets of AD patients

Fišar Z, Hroudová J, Hansíková H, Spáčilová J, Lelková P, Wenchich L, Jirák R, Zvěřová M, Zeman J, Martásek P, Raboch J: Mitochondrial respiration in the platelets of patients with Alzheimer's disease. Current Alzheimer Research 2016; 13(8): 930-941. (IF 2016 = 2.952)

A total of 29 AD patients were recruited for the present study and underwent clinical evaluation and measurements of mitochondrial respiration in blood platelets. Global cognitive impairment in AD patients was characterized and screened by the MMSE (Mini Mental State Exam) score. The control group was recruited from 29 healthy volunteers. Mean values of MMSE, CoQ_{10} concentration and activities of CS and mitochondrial complexes I, II, III, and IV of AD patients and control persons are summarized in Table 10.

Data were evaluated together for women and men because we did not observe a significant difference between women and men in any measured variable. CS activity was used as proper reference value characterizing the amount and potential activity of mitochondria in the sample. Although elderly persons were grouped in controls (above 50 years of age), the mean age of AD patients was significantly higher compared with controls (Table 10).

In the control group, linear regression analysis discovered a significant dependence on age for a few measured respirometric parameters: NetPR/ETSC (p = 0.032) or ROX/ETSC (p = 0.047) in intact platelets and Rot (p = 0.040) in permeabilized platelets. Thus, statistical analysis with a

control for age was performed for all the respirometric data. We did not find a significant association between age and CS activity in elderly controls (p = 0.38). Thus, CS activity was used as proper reference value characterizing the amount and potential activity of mitochondria in the sample.

Table 10 Data of patients with Alzheimer's disease and control subjects.

Characteristic	*Alzheimer's disease*	*Control*
MMSE	***15.3 ± 7.3	29.3 ± 1.0
Complex I (nmol·min^{-1}·mg^{-1})	*47.7 ± 12.7	40.4 ± 15.3
Complex II (nmol·min^{-1}·mg^{-1})	9.0 ± 3.3	8.3 ± 2.5
Complex III (nmol·min^{-1}·mg^{-1})	10.1 ± 5.4	6.2 ± 2.1
Complex IV (nmol·min^{-1}·mg^{-1})	*21.7 ± 4.7	24.4 ± 3.8
CS (nmol·min^{-1}·mg^{-1})	71.0 ± 12.3	76.8 ± 9.5
Complex I/CS	*0.711 ± 0.305	0.535 ± 0.221
Complex II/CS	0.130 ± 0.051	0.108 ± 0.029
Complex III/CS	0.153 ± 0.089	0.085 ± 0.032
Complex IV/CS	0.314 ± 0.080	0.320 ± 0.046
CoQ_{10} (μg·ml^{-1})	***0.53 ± 0.20	0.79 ± 0.23
Age (years)	***78.3 ± 7.2	64.3 ± 6.8
N (women/men)	31 (19/12)	32 (20/12)

The data are displayed as the mean ± SD (N = 29 for all characteristics, except for complex III when N =9); a post-hoc Scheffé test with control for age was used to determine the indicated p-level when compared with controls; *P < 0.05, ***P < 0.001. MMSE, Mini-Mental State Examination score; CoQ_{10}, coenzyme Q_{10}; CS, citrate synthase activity; N, number of participants.

The general linear model and post-hoc Scheffé test with an adjustment for age were used to analyze changes in mitochondrial respiration in the platelets from AD patients compared with controls. The flux control ratio LEAK/ETSC (i.e., oligomycin-inhibited respiration divided by uncoupled respiration at optimum FCCP concentration) in intact platelets remained lower than 0.05, which indicated well coupled mitochondria and the functional integrity of the inner

mitochondrial membrane. In intact platelets, the PR (P = 0.0048) and ETSC (P = 0.0085) decreased significantly in AD patients compared with controls. Respiration after complex I inhibition by rotenone (Rot) was significantly higher in AD (P = 0.013). When normalized for CS activity, differences were significant for Rot/CS (P = 0.0092) but not for PR/CS or ETSC/CS. The fact that CS activity was slightly lower in AD patients compared with controls (Table 10; P = 0.059) was responsible for different results before and after normalizing the respiratory rate relative to CS activity. The flux control ratio, which was determined as the ratio of respiratory rate at a specific respiratory state to ETSC, was significantly higher in AD for Rot/ETSC (P = 0.0030) and ROX/ETSC (P = 0.018) only. In permeabilized platelets, respiration in different respiratory states, as well as flux control ratios, were not significantly different in AD compared with controls, except for lower LEAK (P = 0.046) and LEAK/ETSC (P = 0.022).

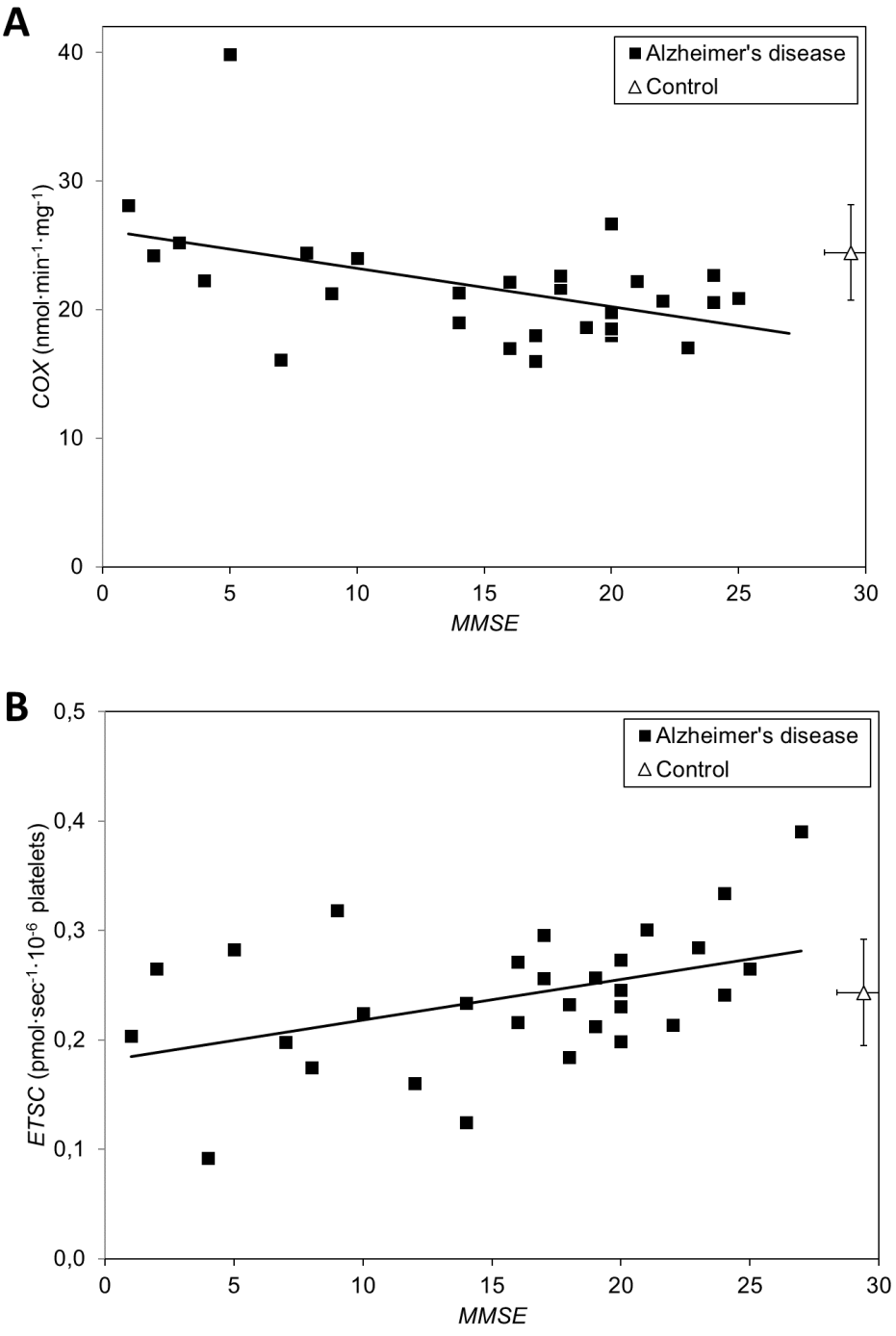
Complexes of respiratory chain and coenzyme Q_{10} content

Significant decrease was found in activity of complex IV in AD patients compared with controls; complex I activity was significantly increased in AD patients. When normalized for CS activity, only the ratio Complex I/CS was significantly different in AD patients compared with controls. Concentration of CoQ_{10} was significantly decreased in AD patients (Table 10).

Correlations between cognitive impairment and mitochondrial parameters

Partial correlation coefficients (with the effect of age removed) between MMSE score and respiratory rate in platelets, CoQ_{10} plasma concentration and activities of respiratory complexes in platelet mitochondria were calculated to evaluate the degree of association between AD progression and mitochondrial variables. Significant partial correlations were found between MMSE and COX (ρ = -0.480, P = 0.013), MMSE and ratio COX/CS (ρ = -0.423, P = 0.028) and MMSE and ETSC in permeabilized platelets (ρ = 0.400, P = 0.039). Dependence of complex IV activity and capacity of ETS in permeabilized platelets on MMSE score is shown on Fig. 2.

Fig. 2 Plots of (A) complex IV activity in platelets (COX), (B) capacity of electron transport system in permeabilized platelets (ETSC) against Mini-Mental State Examination score (MMSE) in patients with Alzheimer's disease. Significant association was found between MMSE and COX (partial correlation coefficient ρ = -0.480; P = 0.013) and between MMSE and ETSC (partial correlation coefficient ρ = 0.400; P = 0.039). Means ± SD (N = 29) are displayed for controls (triangles).



6. Discussion

6.1 Biological models

Pig brain mitochondria

Proper biological models are necessary for a research of mitochondrial functions including the effects of drugs on mitochondrial functions. In isolated pig brain mitochondria, we compared oxygen flux normalized to CS activity and respiration flux control ratios in the crude mitochondrial fraction and mitochondria purified in sucrose gradient or Percoll gradient during long-term storage of samples on ice. Our results indicate that impurities do not significantly interfere with the mitochondrial respirometry analyses. However, contaminants can contribute to the overall protein content. In addition, crude mitochondrial fraction contains synaptosomes, which comprise mitochondria available after digitonin treatment. Nevertheless, gradient purification of mitochondria is not necessary for a number of respirometric measurements.

The mitochondrial parameters of this model are comparable to mitochondria isolated from the liver, muscles, or the brains of the rodents. The advantage of this biological model is the stability of respiratory parameters for more than 48 hours and the possibility of isolating large amounts of mitochondria from specific brain areas. We suggested that high-resolution respirometry of pig brain mitochondria is suitable method for research of the neuroprotective effects and/or mitochondrial toxicity of new medicinal drugs.

Cybrid cells

Cybrid cells were used to study complex I biogenesis during early stages of assembly. Mutations in *MT-ND1* have been described in patients with mitochondrial disease [311], neurodegenerative disorders [312], and some forms of cancer [313]. We originally captured a homoplasmic m.3571dupC mutation in *MT-ND1* in synaptosomal cybrids generated from glioma brain tissue and showed that it abolishes complex I–linked ATP generation [314]. We showed that this mutation results in the total loss of ND1 protein and the disruption complex I assembly at an early stage of its biogenesis. This results in the absence of mature holoenzyme, with an associated loss of complex I–linked mitochondrial respiration.

6.2 Mitochondrial DNA and oxidative phosphorylation

Complex I is a rate limiting enzyme for oxygen consumption in synapses and plays a major role in the control of OXPHOS [315]. Abnormalities in its activity can lead to mitochondrial dysfunction, defects in energy metabolism and thereby to changes in neuronal activity [316], [317]. Moreover, large amounts of ROS are released by defective mitochondria. A decline in antioxidative enzyme activities (e.g. in the elderly) and increase of ROS production lead to neuronal damage due to increase of oxidative stress.

Complex I is a large multimeric enzyme that must be assembled correctly from its 45 subunits to function correctly. This process proceeds via a number of intermediate complexes that are assembled in a coordinated fashion to construct the mature holoenzyme. One of complex I mtDNA encoded subunits, ND1, is incorporated during the early stages of complex I assembly. However, little is known about how mutations in ND1 affect this assembly process. We found that in human 143B cybrid cells carrying a homoplasmic *MT-ND1* mutation, ND1 protein could not be translated. We found that the loss of ND1 stalls complex I assembly during the early stages of its biogenesis, which not only results in the loss of mature complex I but also disrupts the stability of complex IV and the respiratory supercomplex, thus causing mitochondrial dysfunction.

6.3 *In vitro* effects of drugs

6.3.1 Effects of antidepressants and mood stabilizers on mitochondrial respiration

Antidepressants and mood stabilizers are suggested to cause changes in intracellular pathways and affect energy metabolism. Variability in intracellular processes probably participates in interindividual differences in response to antidepressant treatment with or in drug resistance.

CS, the first and rate-limiting enzyme of the TCA cycle, plays a decisive role in regulating energy generation of mitochondrial respiration, complex I is a rate-limiting for oxygen consumption in the synapses [315], and complex IV was suggested as an endogenous metabolic marker for neuronal activity [318]. Therefore, we focused on the study of effects of pharmacologically different antidepressants and mood stabilizers on activities of these mitochondrial enzymes.

Although effect of tested drugs on the activity of CS was not significant, most of the drugs increased CS activity. It is consistent with finding that valproate reversed and lithium prevented amphetamine-induced CS inhibition in animal model [117]. Clearly, drawing from our results, the effect of antidepressants (amitriptyline, fluoxetine, tianeptine) on mitochondrial respiratory rate was inhibitory; the effect of mood stabilizers (lithium, valproate, olanzapine) was negligible. It is consistent with recent findings that mood-stabilizing drugs are able to prevent dysfunctional mitochondrial ETC-induced oxidative damage [319].

Our findings 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 signaling, and if effects of antidepressants and mood stabilizers on mitochondrial functions are related rather to therapeutic or to adverse effects of pharmacotherapy.

Differences between individual drugs with similar physicochemical properties indicate selectivity of drug-induced changes in the mitochondrial respiratory rate. Our findings suggest that mood stabilizers do not interfere with brain mitochondrial respiration, whereas direct mitochondrial targeting is involved in the mechanisms of action of pharmacologically different antidepressants. This indicates the existence of definite selectivity of antidepressant-mitochondria interactions. Antidepressant-induced decrease of respiratory rate can be associated with the adverse effects of antidepressant pharmacotherapy. However, it is obvious that therapeutic effects of antidepressants are associated with long-term adaptive changes in neurotransmission. Thus, it is possible that early antidepressant-induced decrease of respiratory rate is the initial event in a complex cellular response to antidepressants in the intracellular milieu leading to adaptive changes, finally leading to support of neuroplasticity. The hypothesis should be tested that weak antimitochondrial actions of antidepressants could provide a potentially protective pre-conditioning effect [82, 320], in which antidepressant-induced mitochondrial dysfunction below the threshold of injury results in subsequent protection or increase of neuroplasticity.

Further studies of the long-term effects of antidepressants and mood stabilizers on the molecular level are necessary to understand their roles in signaling pathways and influences on the energy metabolism of neurons. They are expected to be helpful in the search for biological

markers of mood disorders, predictors of efficiency of antidepressant treatment and in the search for new psychotropics.

6.3.2 Effects of cognitives and nootropics on mitochondrial respiration

Effects of drugs used for symptomatic AD treatment may be related to their direct action on mitochondrial function. The *in vitro* effects of pharmacologically different drugs on selected mitochondrial parameters were investigated: activities of ETC complexes I, II + III, and IV, CS, MAO, oxygen consumption rate, and H_2O_2 production in pig brain mitochondria. The direct effect of cognitives and nootropics used in the treatment of AD on mitochondrial respiration is relatively small. We observed mild complex I inhibition by galantamine, donepezil, and memantine, indicating the possible involvement of mitochondrial dysfunction on the adverse effects of high doses of these drugs. The safest drugs in terms of disturbing mitochondrial function appear to be piracetam and rivastigmine. The results support future research aimed at measuring the effects of currently used drugs or newly synthesized drugs on mitochondrial functioning in order to understand their mechanism of action.

Respiratory complexes I and III are taken as the source of the most ROS in mitochondria, and ROS generation and release can be regulated by the choice of substrates and inhibitors. During complex I-linked respiration, ROS are produced from complexes I and III. Complex II-linked respiration leads to ROS production to some extent by complex III but also through reverse electron flow to complex I. Electron flow through the complexes can be inhibited selectively, e.g., by rotenone (complex I) and antimycin A (complex III), resulting in an alteration in the production of ROS [321]. We measured the drug's effect on the release of mitochondrial H_2O_2 in the presence of succinate + rotenone + antimycin A. Although such ROS production is non-physiological, we confirmed that it can be used to evaluate the protective effect of cognitives and nootropics on mitochondrial ROS production.

6.3.3 *In vitro* evaluation new tacrine derivatives, AChE inhibitors and oximes

We examined the effect of selected inhibitors (tacrine, 7-MEOTA) and reactivators of AChE (pralidoxime, trimedoxime, obidoxime, methoxime, HI-6) on the complex I activity. It can be concluded that the extent of inhibition is relatively small compared to known complex I inhibitors

such as conventional antipsychotics, and the oxime interaction with complex I seems not to be clinically significant. Drug concentration used in our experiment was near to maximal expected brain concentration of oximes.

Results obtained with AChE inhibitors confirm that 7-MEOTA do not significantly inhibit NADH dehydrogenase activity. Tacrine was the only significant inhibitor of complex I in our study. Tacrine shows various adverse effects. In study with human hepatoma cell line Hep G2, tacrine caused increase of the TCA cycle, which could be a signature of uncoupling of the OXPHOS [322]. Another study compared parameters leading to oxidative stress – differences were found between newly developed AChE inhibitor PMS777 and tacrine; PMS777 was able to fight inflammatory event whereas tacrine was able to minimize them [323]. It was shown that tacrine induces cytotoxicity both via inhibition of mitochondrial energization and by destabilization of membrane phospholipids associated with oxidative stress [324]. In our study, drug concentrations were higher (50 μmol/l); in spite of this fact, other drugs tested than tacrine have not influenced significantly Complex I activity.

6.4 *In vitro* effects of drugs on MAO isoforms

MAO catalyzes the oxidative deamination of a variety of biogenic and xenobiotic amines with concomitant H_2O_2 production. MAO activity is inhibited by many psychotropic drugs [112]; and MAO inhibitors are considered potential candidates for AD treatment, due to their ability to inhibit oxidative damage [282, 290].

6.4.1 Effects of antidepressants and mood stabilizers

The experimental conditions of our study, the use of the selective substrates for MAO-A and MAO-B, allowed to evaluate inhibitory effects and mechanism of action of antidepressants and mood stabilizers on the two MAO isoforms separately [172, 325]. 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 *Ki* 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 different antidepressants or mood stabilizers could act, at least in part, by inhibition of MAO.

The study confirmed and extended previous results [153, 160, 161, 326] that antidepressant drugs of various pharmacological classes (tricyclic antidepressants, SSRIs, NRIs, SNRIs, NaSSAs, NDRIs, SSRE) show inhibitory effects on MAOs. All tested antidepressants and mood stabilizers, except for venlafaxine and olanzapine, 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 0.11 for imipramine to 0.89 for tianeptine). Different IC_{50} or *Ki* 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.

Since MAO is embedded in the outer mitochondrial membrane, lipid-protein interactions play a role in the functional properties of MAOs [327, 328], and antidepressants are capable of changing membrane fluidity, it can be speculated about a role of accumulation of antidepressants in the lipid bilayer related to their effects on MAO activity. However, some data indicate that fluidity modulation is not the cause of MAO inhibition [329]. 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 serotonin was used as substrate. Competitive inhibition would be consistent with drug binding to the active sites of the MAO-A. Desipramine, amitriptyline, imipramine, mirtazapine and tianeptine retain the same mechanism of inhibition for both MAO-A and MAO-B.

Antidepressants inhibited MAOs at concentrations much higher than therapeutically active plasma concentrations of antidepressants *in vivo*. However, there is an accumulation of psychotropic drugs in the brain and the brain levels of antidepressants are a reflection not only of their plasma concentrations, but also of their distribution between plasma and red blood cells [330, 331]. 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 for up to 35 μmol/l [332]. It has also been shown that fluoxetine is present in subcellular components of the rodent brain, including mitochondria and synaptosomes [333]. 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.

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 [172]. 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 still unclear; however, it is obvious that reduction of local oxidative stress is a major component, and MAO inhibition participates in this reduction.

6.4.2 Effects of cognitives and nootropics

While MAO inhibition is not a primary biochemical effect related to therapeutic action of cognitives and nootropics, it can be supposed that changes in MAO activity may be related to some of the effects of these drugs on serotonergic, noradrenergic, and dopaminergic neurotransmission. Moreover, MAO inhibition may participate in antioxidant and neuroprotective effects of cognitives and nootropics. We found that the most potent MAO inhibitors are latrepirdine, 7-MEOTA, and donepezil. The IC_{50} of approximately 5 μmol/L for inhibition of MAO-A indicates that MAO-A activity may be inhibited at therapeutic doses of these drugs. Thus, neuroprotective and antidepressant or mood altering effects can be of added value to the improved cognition by treatment with these drugs. Latrepirdine, 7-MEOTA, donepezil, and memantine were found to be selective MAO-A inhibitors (Table 7), which is in conflict with previous findings that latrepirdine in rat brain homogenate [334] and donepezil in rat liver homogenate [290] preferentially inhibited MAO-B. We suppose that the purification of mitochondria may play a role in this discrepancy because the presence of non-mitochondrial membranes may affect the concentrations of free substances. The lowest potency to inhibit MAO-A was found for piracetam; however, 1 mmol/L piracetam plasma concentration may be reached during therapy, which is sufficient to partially inhibit MAO-A. In contrast, a stimulatory effect on MAO-B was found for piracetam. These findings indicate that the resulting effect of piracetam on MAO activity may be different in various tissues/cells with different representations of MAO-A and MAO-B [335].

6.4.3 Effects of acetylcholinesterase reactivators

Several studies have shown that oximes might have additional pharmacological effects, other than AChE reactivation. It is doubtful if they contribute significantly to lifesaving action of oximes relative to the reactivation of AChE; however, they could participate in therapeutic and/or adverse effects of oximes in the CNS. The study on the interaction of oximes with MAO is important for prediction of potential influence of oximes on monoaminergic neurotransmission in the CNS, i.e. on processes related to psychiatric or neurodegenerative disorders.

Our results show that all tested oximes are full inhibitors of the catalytic site of MAO-A for serotonin, although with low potency. However, only HI-6 and pralidoxime are able to inhibit also the catalytic site of MAO-B for PEA. The effective dose of oximes reaching the half maximal MAO-A activity was in the rage of 0.4–5.0 mmol/l. Similarly, inhibition of MAO-B by HI-6 and pralidoxime occurs at high drug concentrations, too. These oxime concentrations could be hardly attainable in the brain *in vivo*. It was shown that various oximes penetrate the blood–brain barrier and their brain concentrations reach about 5–10% of their plasma concentrations [336]. Since plasma concentrations are limited to about 10−4 mol/l by the inherent acute toxicity of oximes [337], expected maximal brain concentrations are about 10−5 mol/l. There is no significant *in vitro* effect on MAO activity at these oxime concentrations. However, intraneuronal oximes concentrations are not known.

Although it is always difficult to extrapolate from *in vitro* studies to the clinical reality, our results suggest that the examined oximes show weak MAO-A inhibition and very weak inhibition or multiphase effects on MAO-B activity. Present results indicate that pralidoxime, obidoxime, trimedoxime, methoxime and HI-6 can be taken as the relatively safe compounds regarding to drug-induced changes in MAO activity and to related changes in monoaminergic neurotransmission; however, a systematic *in vivo* investigation of the oximes effects on MAO activity is necessary to confirm the clinically important conclusion.

6.5 Mitochondrial respiration in neuropsychiatric diseases

The methodology of *in vitro* measurements has been consequently modified to measure the same mitochondrial parameters (respiratory rate and mitochondrial enzyme activities) in blood platelets of psychiatric patients. Analysis of data enables us to determine suitable mitochondrial

parameters, which are measurable in peripheral blood and could be further studied as potential biological markers of psychiatric disorders.

Results from respirometry measurements correlate with spectrophotometric measurements. Increased physiological respiration of mitochondria reflects increase of complex I activity, decreased respiratory rate after complex I inhibition reflects decrease of complex II activity. It can be presumed that decrease in complex II activity is caused by adaptive mechanisms as a result of increased complex I activity. Therefore, it is necessary to determine whether mitochondrial dysfunctions are a cause or a result of abnormal signalization.

Platelets from peripheral blood contribute very little to the overall metabolic turnover; however, the respiratory rate might serve as an easily available biological marker for studying the changes of mitochondrial function. However, experimental protocols have been developed to allow standardized measurement of mitochondrial respiration in human blood samples in search for diagnostic biomarkers and/or monitoring the effects of pharmacotherapy. We suppose that platelets are a suitable blood-based model for studying mitochondrial respiration due to the simple isolation of intact platelets, which enables *in situ* measurements. Moreover, low platelet sensitivity may be expected toward the mechanisms of cellular compensation that are induced by the disease; these compensatory processes supply the energy required for cellular functioning and can lead to an increase in ETS activity in lymphocytes [210].

6.5.1 Depressive disorder

We examined alterations of mitochondrial respiration in blood platelets of patients with current depressive episode in the presence of mild to severe depressive symptoms before and after treatment. We found that both physiological respiratory rate and maximal capacity of ETS in intact platelets were significantly decreased in patients with a diagnosis of depressive disorder, which were in remission or partial remission, when compared to controls. These parameters were unchanged, when respiratory rate was normalized for CS activity. No changes in measured mitochondrial parameters were observed in permeabilized platelets. Thus, intact but not permeabilized platelets could be an appropriate biological model for the measurement of respiratory rate (PR or ETS capacity) as a biological marker of depressive disorder.

6.5.2 Bipolar disorder

We did not observe significant differences in individual ETS complex activities in platelets of BD patients, similarly to the study, when activities of ETS complexes were examined in mononuclear cells of BD patients [338]. We found a significantly increased ratio of complex I to CS activity. However, the complex I/CS ratio cannot be used as a specific marker for BD, because an increased complex I/CS ratio was also observed in AD patients [210, 339] and increased complex I activity was also seen in blood platelets from patients with anorexia nervosa [340].

In intact platelets, the examined mitochondrial respiratory rates PR, ETSC and LEAK were increased, and Rot was decreased. We hypothesize that increased complex I activity in BD with manic episode may be an adaptive mechanism responsible for both increased physiological mitochondrial respiration in platelets and for decreased mitochondrial respiration after complex I inhibition. Disturbances in complex I activity are also related to ROS production by mitochondria and participate in cell damage due to oxidative stress. Our results support the view that the pathology of complex I corresponds with the diversity and similarities in clinical symptoms of various psychiatric and neurodegenerative disorders [341, 342].

In permeabilized platelets of BD patients, after the supplementation of both NADH- and $FADH_2$ (reduced flavin adenine dinucleotide) linked substrates, ETSC was found to be significantly decreased. Substrates were added in excess, thus, decrease of ETS capacity is probably not caused by insufficient availability of substrates and coenzymes entering into OXPHOS. It can be more likely explained by the deficient function of enzymes in the citric acid cycle and OXPHOS. We can speculate that the observed increase in complex I activity in BD patients could be a compensatory mechanism, which ensures unchanged ETSC in intact platelets.

6.5.3 Alzheimer's disease

We found that both PR (endogenous basal rate of respiration) and the ETSC in intact platelets decreased significantly in AD patients compared with controls. It was an important finding that respiration in intact platelets but not in permeabilized platelets is associated with AD. However, the CS normalization eliminated the significant difference in physiological respiration and ETSC, suggesting that the observed differences in the physiological respiration and maximal capacity

are slight and cannot be used as a biological marker of AD. The respirometry parameters, which were significantly changed in AD compared with controls, were not age-dependent. Thus, although the mean age was significantly higher in the AD patient group compared with controls, differences in respiratory rates that were observed between AD patients and controls could be attributed to the effect of the disease. Statistical analysis with a control for the confounding effect of age was performed and confirmed this statement.

Conclusions

Biological markers of neuropsychiatric diseases, predictors of the response to drug administration and molecular targets of newly developed drugs are researched on the basis of recent hypotheses of psychiatric and neurodegenerative disorders.

The presumption was confirmed that both weak mitochondrial dysfunctions and interactions between psychopharmaca and OXPHOS can significantly influence intracellular processes related to psychiatric disorders. *In vitro* results from our measurements and analyses revealed to what extent mitochondrial functions are sensitive to the early effects of antidepressants, mood stabilizers and other psychotropic drugs.

Differences in inhibitory potency and in mechanism of inhibition have been found between different psychotropic drugs. While mitochondrial respiratory rate was inhibited at higher concentrations of antidepressants, it was not affected by mood stabilizers, olanzapine or ketamine. 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. Antidepressants and mood stabilizers tested did not significantly change CS activity. Activity of ETC complexes was decreased, complexes I and IV being the most affected.

Supposing that the mechanism of action of antidepressants and mood stabilizers is related to processes implicated in the 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 suitable candidates in searching for new biological markers of mood disorders, targets of new antidepressants or predictors of response to pharmacotherapy.

Changes in mitochondrial parameters, 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 in 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 signaling, and if the effects of antidepressants and mood stabilizers on mitochondrial functions are related rather to therapeutic effects or to the adverse effects of pharmacotherapy.

The different *in vitro* effects of piracetam, latrepirdine, 7-MEOTA, galantamine, donepezil, rivastigmine, and memantine on mitochondrial respiration, the enzymatic activity of CS and ETC complexes, H_2O_2 production, and MAO activity support the presumed hypothesis for the independent mode of mitochondrial action of various cognitives and nootropics. We confirmed that the cognitives and nootropics utilized in this study have a relatively small direct early effect on selected mitochondrial functions, even at high concentrations. The high drug concentration required for this direct mitochondrial effect indicates that non-receptor mechanisms play a role. The inhibitory effects of these drugs on MAO-A may participate in mitochondrial neuroprotection; however, they should be taken into account if other drugs affecting monoaminergic transmission are coadministered. Drug-induced changes in mitochondrial functions should be included in a panel of tests for newly synthesized drugs in order to rule out their mitochondrial toxicity and have a better understanding of their mechanisms of action.

Recently, novel tacrine and 7-MEOTA derivatives were synthesized and extensively investigated to find less toxic compounds affecting more AD pathological mechanisms. There is less known about the effects of these drugs on mitochondrial functions and cellular energy metabolism. The effect of newly synthesized psychotropic drugs on selected mitochondrial parameters should be included in their testing to discover their mitochondrial toxicity and/or potential neurotrophic effects.

Impaired functions of mitochondria seem to contribute to a wide range of diseases; affective disorders as well as neurodegenerative diseases. We confirmed that changes in the rate of oxygen consumption might participate in the pathophysiology of depression. Decreased physiological respiration and ETS capacity in depressive subjects might be related to the depressive disorder; however, study of mitochondrial respiration in drug-naïve depressive patients before and after treatment is necessary to distinguish reliably the effect of the disorder from the effect of antidepressants. Our results support the hypothesis that respiratory rate is a nonspecific marker of depressive disorder. Thus, both decreased availability of substrates or coenzymes of OXPHOS and decreased activity of complex II and other components of OXPHOS may underlie vulnerability to depression. It remains to be determined if mitochondrial dysfunction is rather a causal or a consequential event of abnormal signaling in depressive disorder.

The changes in the ETS complex activities and mitochondrial oxygen consumption rate support the hypothesis that impaired energy metabolism might participate in BD pathophysiology. The results obtained evidence that energy metabolism in blood platelets of BD patients differs from healthy controls. Measurements in permeabilized platelets indicate impaired function of OXPHOS system in BD patients in a manic episode. Measurement of the activity of ETS complexes and measurement of mitochondrial respiration in intact platelets indicate complex I-enhanced bioenergy as an adaptive response to this damage. In conclusion, better insight into molecular mechanisms of cellular respiration could lead to better understanding of pathophysiology of BD and it is reasonable to study mitochondrial dysfunctions in different episodes of BD. Mitochondrial dysfunctions in different episodes of BD should be further studied.

We demonstrated a decrease in the respiratory rate in intact platelets but not in permeabilized platelets of AD patients. When normalized for CS activity the only thing that remained significantly altered was the respiratory rate after inhibiting complex I of ETS. A significant correlation between MMSE score and ETS capacity in permeabilized platelets indicate that cognitive impairment in AD is associated with a disturbance in cellular energetics. Complex I activity was increased, complex IV activity was decreased, and coenzyme Q_{10} plasma concentration was decreased in AD. Therefore, it can be presumed that disturbed mitochondrial respiration in AD is caused both by processes that threaten neuronal energy metabolism at the level of input into OXPHOS and by the impaired function of ETS complexes, mainly COX. The relatively greater utilization of complex II input into the ETS in the intact platelets from AD patients as well as changes in complex I activity may be compensatory processes for a disturbance in mitochondrial functions. Complex I activity seems to be a potential marker for AD. Our data suggest that intact platelets are easily available components of peripheral blood, which might be useful for *in situ* studies of mitochondrial respiration in neurodegenerative disorders.

References

1. Shao, L., et al., *Mitochondrial involvement in psychiatric disorders.* Ann Med, 2008. **40**(4): p. 281-95.
2. Rezin, G.T., et al., *Mitochondrial dysfunction and psychiatric disorders.* Neurochem Res, 2009. **34**(6): p. 1021-9.
3. Jou, S.H., N.Y. Chiu, and C.S. Liu, *Mitochondrial dysfunction and psychiatric disorders.* Chang Gung Med J, 2009. **32**(4): p. 370-9.
4. Orth, M. and A.H. Schapira, *Mitochondria and degenerative disorders.* Am J Med Genet, 2001. **106**(1): p. 27-36.
5. Schapira, A.H., *Mitochondrial diseases.* Lancet, 2012. **379**(9828): p. 1825-34.
6. Fattal, O., et al., *Review of the literature on major mental disorders in adult patients with mitochondrial diseases.* Psychosomatics, 2006. **47**(1): p. 1-7.
7. Washizuka, S., et al., *Association of mitochondrial complex I subunit gene NDUFV2 at 18p11 with schizophrenia in the Japanese population.* Am J Med Genet B Neuropsychiatr Genet, 2006. **141b**(3): p. 301-4.
8. Kato, T., et al., *Mitochondrial DNA polymorphisms in bipolar disorder.* J Affect Disord, 2001. **62**(3): p. 151-64.
9. Iwamoto, K., M. Bundo, and T. Kato, *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): p. 241-53.
10. Pittenger, C. and R.S. Duman, *Stress, depression, and neuroplasticity: a convergence of mechanisms.* Neuropsychopharmacology, 2008. **33**(1): p. 88-109.
11. Maes, M., et al., *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): p. 127-50.
12. Fisar, Z. and J. Raboch, *Depression, antidepressants, and peripheral blood components.* Neuro Endocrinol Lett, 2008. **29**(1): p. 17-28.
13. Fisar, Z. and J. Hroudova, *Intracellular signalling pathways and mood disorders*, in *Folia Biol (Praha)*. 2010: Czech Republic. p. 135-48.
14. Duman, R.S., G.R. Heninger, and E.J. Nestler, *A molecular and cellular theory of depression.* Arch Gen Psychiatry, 1997. **54**(7): p. 597-606.
15. Duman, R.S., *Synaptic plasticity and mood disorders.* Mol Psychiatry, 2002. **7 Suppl 1**: p. S29-34.
16. Zarate, C.A., Jr., J. Singh, and H.K. Manji, *Cellular plasticity cascades: targets for the development of novel therapeutics for bipolar disorder.* Biol Psychiatry, 2006. **59**(11): p. 1006-20.
17. Einat, H. and H.K. Manji, *Cellular plasticity cascades: genes-to-behavior pathways in animal models of bipolar disorder.* Biol Psychiatry, 2006. **59**(12): p. 1160-71.
18. Stork, C. and P.F. Renshaw, *Mitochondrial dysfunction in bipolar disorder: evidence from magnetic resonance spectroscopy research.* Mol Psychiatry, 2005. **10**(10): p. 900-19.
19. Kato, T., *Role of mitochondrial DNA in calcium signaling abnormality in bipolar disorder.* Cell Calcium, 2008. **44**(1): p. 92-102.

20. Quiroz, J.A., et al., *Mitochondrially mediated plasticity in the pathophysiology and treatment of bipolar disorder.* Neuropsychopharmacology, 2008. **33**(11): p. 2551-65.
21. Fišar, Z., *Biochemické hypotézy afektivních poruch.* Galén, Praha, 1998.
22. Fišar, Z., et al., *Vybrané kapitoly z biologické psychiatrie, 2. přepracované a doplněné vydání.* Grada Publishing, a.s., Praha, 2009.
23. Krishnan, V. and E.J. Nestler, *The molecular neurobiology of depression.* Nature, 2008. **455**(7215): p. 894-902.
24. Mathew, S.J., H.K. Manji, and D.S. Charney, *Novel drugs and therapeutic targets for severe mood disorders.* Neuropsychopharmacology, 2008. **33**(9): p. 2080-92.
25. aan het Rot, M., S.J. Mathew, and D.S. Charney, *Neurobiological mechanisms in major depressive disorder.* Cmaj, 2009. **180**(3): p. 305-13.
26. Coppen, A., *The biochemistry of affective disorders.* Br J Psychiatry, 1967. **113**(504): p. 1237-64.
27. Schildkraut, J.J., *The catecholamine hypothesis of affective disorders: a review of supporting evidence.* Am J Psychiatry, 1965. **122**(5): p. 509-22.
28. Nestler, E.J., et al., *Neurobiology of depression.* Neuron, 2002. **34**(1): p. 13-25.
29. Hindmarch, I., *Beyond the monoamine hypothesis: mechanisms, molecules and methods.* Eur Psychiatry, 2002. **17 Suppl 3**: p. 294-9.
30. Delgado, P.L., et al., *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): p. 212-20.
31. Ruhe, H.G., N.S. Mason, and A.H. Schene, *Mood is indirectly related to serotonin, norepinephrine and dopamine levels in humans: a meta-analysis of monoamine depletion studies.* Mol Psychiatry, 2007. **12**(4): p. 331-59.
32. Cowen, P.J., *Serotonin and depression: pathophysiological mechanism or marketing myth?* Trends Pharmacol Sci, 2008. **29**(9): p. 433-6.
33. Mendelsohn, D., W.J. Riedel, and A. Sambeth, *Effects of acute tryptophan depletion on memory, attention and executive functions: a systematic review.* Neurosci Biobehav Rev, 2009. **33**(6): p. 926-52.
34. Berman, R.M., et al., *Monoamine depletion in unmedicated depressed subjects.* Biol Psychiatry, 2002. **51**(6): p. 469-73.
35. Heninger, G.R., P.L. Delgado, and D.S. Charney, *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): p. 2-11.
36. Jacobs, B.L., H. van Praag, and F.H. Gage, *Adult brain neurogenesis and psychiatry: a novel theory of depression.* Mol Psychiatry, 2000. **5**(3): p. 262-9.
37. Jacobs, B.L., *Adult brain neurogenesis and depression.* Brain Behav Immun, 2002. **16**(5): p. 602-9.
38. Kempermann, G. and G. Kronenberg, *Depressed new neurons--adult hippocampal neurogenesis and a cellular plasticity hypothesis of major depression.* Biol Psychiatry, 2003. **54**(5): p. 499-503.
39. Kato, T. and N. Kato, *Mitochondrial dysfunction in bipolar disorder.* Bipolar Disord, 2000. **2**(3 Pt 1): p. 180-90.
40. Ben-Shachar, D. and R. Karry, *Neuroanatomical pattern of mitochondrial complex I pathology varies between schizophrenia, bipolar disorder and major depression.* PLoS One, 2008. **3**(11): p. e3676.

41. Gardner, A. and R.G. Boles, *Beyond the serotonin hypothesis: mitochondria, inflammation and neurodegeneration in major depression and affective spectrum disorders.* Prog Neuropsychopharmacol Biol Psychiatry, 2011. **35**(3): p. 730-43.
42. Gardner, A., et al., *Alterations of mitochondrial function and correlations with personality traits in selected major depressive disorder patients.* J Affect Disord, 2003. **76**(1-3): p. 55-68.
43. Gardner, A. and R.G. Boles, *Symptoms of somatization as a rapid screening tool for mitochondrial dysfunction in depression.* Biopsychosoc Med, 2008. **2**: p. 7.
44. Andreazza, A.C., et al., *Mitochondrial complex I activity and oxidative damage to mitochondrial proteins in the prefrontal cortex of patients with bipolar disorder.* Arch Gen Psychiatry, 2010. **67**(4): p. 360-8.
45. Haddad, J.J., N.E. Saade, and B. Safieh-Garabedian, *Cytokines and neuro-immune-endocrine interactions: a role for the hypothalamic-pituitary-adrenal revolving axis.* J Neuroimmunol, 2002. **133**(1-2): p. 1-19.
46. Leonard, B.E. and A. Myint, *The psychoneuroimmunology of depression.* Hum Psychopharmacol, 2009. **24**(3): p. 165-75.
47. Catena-Dell'Osso, M., et al., *Inflammatory and neurodegenerative pathways in depression: a new avenue for antidepressant development?* Curr Med Chem, 2011. **18**(2): p. 245-55.
48. Maes, M., et al., *The inflammatory & neurodegenerative (I&ND) hypothesis of depression: leads for future research and new drug developments in depression.* Metab Brain Dis, 2009. **24**(1): p. 27-53.
49. Judd, L.L., et al., *Residual symptom recovery from major affective episodes in bipolar disorders and rapid episode relapse/recurrence.* Arch Gen Psychiatry, 2008. **65**(4): p. 386-94.
50. Muller-Oerlinghausen, B., A. Berghofer, and M. Bauer, *Bipolar disorder.* Lancet, 2002. **359**(9302): p. 241-7.
51. Berk, M., et al., *Pathways underlying neuroprogression in bipolar disorder: focus on inflammation, oxidative stress and neurotrophic factors.* Neurosci Biobehav Rev, 2011. **35**(3): p. 804-17.
52. Savitz, J. and W.C. Drevets, *Bipolar and major depressive disorder: neuroimaging the developmental-degenerative divide.* Neurosci Biobehav Rev, 2009. **33**(5): p. 699-771.
53. Hamon, M. and P. Blier, *Monoamine neurocircuitry in depression and strategies for new treatments.* Prog Neuropsychopharmacol Biol Psychiatry, 2013. **45**: p. 54-63.
54. Hillhouse, T.M. and J.H. Porter, *A brief history of the development of antidepressant drugs: from monoamines to glutamate.* Exp Clin Psychopharmacol, 2015. **23**(1): p. 1-21.
55. Fisar, Z., *Drugs related to monoamine oxidase activity.* Prog Neuropsychopharmacol Biol Psychiatry, 2016. **69**: p. 112-24.
56. Craddock, N., S. Dave, and J. Greening, *Association studies of bipolar disorder.* Bipolar Disord, 2001. **3**(6): p. 284-98.
57. Serretti, A. and L. Mandelli, *The genetics of bipolar disorder: genome 'hot regions,' genes, new potential candidates and future directions.* Mol Psychiatry, 2008. **13**(8): p. 742-71.
58. Rivera, M., et al., *High-activity variants of the uMAOA polymorphism increase the risk for depression in a large primary care sample.* Am J Med Genet B Neuropsychiatr Genet, 2009. **150b**(3): p. 395-402.

59. Meyer, J.H., et al., *Elevated monoamine oxidase a levels in the brain: an explanation for the monoamine imbalance of major depression.* Arch Gen Psychiatry, 2006. **63**(11): p. 1209-16.
60. Meyer, J.H., et al., *Brain monoamine oxidase A binding in major depressive disorder: relationship to selective serotonin reuptake inhibitor treatment, recovery, and recurrence.* Arch Gen Psychiatry, 2009. **66**(12): p. 1304-12.
61. Selvaraj, S., et al., *Diminished brain 5-HT transporter binding in major depression: a positron emission tomography study with [11C]DASB.* Psychopharmacology (Berl), 2011. **213**(2-3): p. 555-62.
62. Kato, T., *Mitochondrial dysfunction as the molecular basis of bipolar disorder: therapeutic implications.* CNS Drugs, 2007. **21**(1): p. 1-11.
63. Berk, M., et al., *Dopamine dysregulation syndrome: implications for a dopamine hypothesis of bipolar disorder.* Acta Psychiatr Scand Suppl, 2007(434): p. 41-9.
64. Dunlop, B.W. and C.B. Nemeroff, *The role of dopamine in the pathophysiology of depression.* Arch Gen Psychiatry, 2007. **64**(3): p. 327-37.
65. Rao, J.S., et al., *Dysregulated glutamate and dopamine transporters in postmortem frontal cortex from bipolar and schizophrenic patients.* J Affect Disord, 2012. **136**(1-2): p. 63-71.
66. Cannon, D.M., et al., *Reduced muscarinic type 2 receptor binding in subjects with bipolar disorder.* Arch Gen Psychiatry, 2006. **63**(7): p. 741-7.
67. Cannon, D.M., et al., *Genetic variation in cholinergic muscarinic-2 receptor gene modulates M2 receptor binding in vivo and accounts for reduced binding in bipolar disorder.* Mol Psychiatry, 2011. **16**(4): p. 407-18.
68. Lan, M.J., et al., *Metabonomic analysis identifies molecular changes associated with the pathophysiology and drug treatment of bipolar disorder.* Mol Psychiatry, 2009. **14**(3): p. 269-79.
69. Gigante, A.D., et al., *Brain glutamate levels measured by magnetic resonance spectroscopy in patients with bipolar disorder: a meta-analysis.* Bipolar Disord, 2012. **14**(5): p. 478-87.
70. Sanacora, G., et al., *Targeting the glutamatergic system to develop novel, improved therapeutics for mood disorders.* Nat Rev Drug Discov, 2008. **7**(5): p. 426-37.
71. Jun, C., et al., *Disturbance of the glutamatergic system in mood disorders.* Exp Neurobiol, 2014. **23**(1): p. 28-35.
72. Gerhard, D.M., E.S. Wohleb, and R.S. Duman, *Emerging treatment mechanisms for depression: focus on glutamate and synaptic plasticity.* Drug Discov Today, 2016. **21**(3): p. 454-64.
73. Machado-Vieira, R., I.D. Henter, and C.A. Zarate, Jr., *New targets for rapid antidepressant action.* Prog Neurobiol, 2017. **152**: p. 21-37.
74. Duman, R.S., *Depression: a case of neuronal life and death?* Biol Psychiatry, 2004. **56**(3): p. 140-5.
75. Brand, M.D. and D.G. Nicholls, *Assessing mitochondrial dysfunction in cells.* Biochem J, 2011. **435**(2): p. 297-312.
76. Weinbach, E.C., et al., *Effects of tricyclic antidepressant drugs on energy-linked reactions in mitochondria.* Biochem Pharmacol, 1986. **35**(9): p. 1445-51.
77. Katyare, S.S. and R.R. Rajan, *Effect of long-term in vivo treatment with imipramine on the oxidative energy metabolism in rat brain mitochondria.* Comp Biochem Physiol C Pharmacol Toxicol Endocrinol, 1995. **112**(3): p. 353-7.

78. Katyare, S.S. and R.R. Rajan, *Enhanced oxidative phosphorylation in rat liver mitochondria following prolonged in vivo treatment with imipramine.* Br J Pharmacol, 1988. **95**(3): p. 914-22.
79. Xia, Z., et al., *Tricyclic antidepressants induce apoptosis in human T lymphocytes.* Int J Immunopharmacol, 1997. **19**(11-12): p. 645-54.
80. Karlsson, H., et al., *Induction of apoptosis in proliferating lymphocytes by tricyclic antidepressants.* Apoptosis, 1998. **3**(4): p. 255-60.
81. Xia, Z., et al., *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): p. 1199-208.
82. Abdel-Razaq, W., D.A. Kendall, and T.E. Bates, *The Effects of Antidepressants on Mitochondrial Function in a Model Cell System and Isolated Mitochondria.* Neurochemical Research, 2011. **36**(2): p. 327-338.
83. Fromenty, B., et al., *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): p. 3743-51.
84. Souza, M.E., et al., *Effect of fluoxetine on rat liver mitochondria.* Biochem Pharmacol, 1994. **48**(3): p. 535-41.
85. Curti, C., et al., *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): p. 103-9.
86. Han, Y.S. and C.S. Lee, *Antidepressants reveal differential effect against 1-methyl-4-phenylpyridinium toxicity in differentiated PC12 cells.* Eur J Pharmacol, 2009. **604**(1-3): p. 36-44.
87. Kolla, N., et al., *Amitriptyline and fluoxetine protect PC12 cells from cell death induced by hydrogen peroxide.* J Psychiatry Neurosci, 2005. **30**(3): p. 196-201.
88. Wang, H., et al., *Nortriptyline delays disease onset in models of chronic neurodegeneration.* Eur J Neurosci, 2007. **26**(3): p. 633-41.
89. Zhang, W.H., et al., *Nortriptyline protects mitochondria and reduces cerebral ischemia/hypoxia injury.* Stroke, 2008. **39**(2): p. 455-62.
90. Morota, S., et al., *Evaluation of putative inhibitors of mitochondrial permeability transition for brain disorders--specificity vs. toxicity.* Exp Neurol, 2009. **218**(2): p. 353-62.
91. Agostinho, F.R., et al., *Olanzapine plus fluoxetine treatment alters mitochondrial respiratory chain activity in the rat brain.* Acta Neuropsychiatrica, 2011. **23**(6): p. 282-291.
92. Dykens, J.A., et al., *In vitro assessment of mitochondrial dysfunction and cytotoxicity of nefazodone, trazodone, and buspirone.* Toxicol Sci, 2008. **103**(2): p. 335-45.
93. Velasco, A., et al., *Effect of trazodone on oxidative metabolism of rat brain in vitro.* Rev Esp Fisiol, 1985. **41**(2): p. 201-5.
94. Gamaro, G.D., et al., *Reduction of hippocampal Na+, K+-ATPase activity in rats subjected to an experimental model of depression.* Neurochem Res, 2003. **28**(9): p. 1339-44.
95. Li, Y., et al., *Mitochondrial dysfunction induced by sertraline, an antidepressant agent.* Toxicol Sci, 2012. **127**(2): p. 582-91.

96. Scaini, G., et al., *Evaluation of Krebs cycle enzymes in the brain of rats after chronic administration of antidepressants.* Brain Res Bull, 2010. **82**(3-4): p. 224-7.
97. Levkovitz, Y., et al., *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): p. 29-42.
98. Ma, J., et al., *Desipramine induces apoptosis in rat glioma cells via endoplasmic reticulum stress-dependent CHOP pathway.* J Neurooncol, 2011. **101**(1): p. 41-8.
99. Gould, T.D. and H.K. Manji, *Glycogen synthase kinase-3: a putative molecular target for lithium mimetic drugs.* Neuropsychopharmacology, 2005. **30**(7): p. 1223-37.
100. Shaltiel, G., G. Chen, and H.K. Manji, *Neurotrophic signaling cascades in the pathophysiology and treatment of bipolar disorder.* Curr Opin Pharmacol, 2007. **7**(1): p. 22-6.
101. Gould, T.D., et al., *Emerging experimental therapeutics for bipolar disorder: insights from the molecular and cellular actions of current mood stabilizers.* Mol Psychiatry, 2004. **9**(8): p. 734-55.
102. Chen, G., et al., *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): p. 879-82.
103. Bown, C.D., et al., *Regulation of ER stress proteins by valproate: therapeutic implications.* Bipolar Disord, 2002. **4**(2): p. 145-51.
104. Chuang, D.M., *The antiapoptotic actions of mood stabilizers: molecular mechanisms and therapeutic potentials.* Ann N Y Acad Sci, 2005. **1053**: p. 195-204.
105. Magder, D.M., I. Aleksic, and S.H. Kennedy, *Tolerability and efficacy of high-dose moclobemide alone and in combination with lithium and trazodone.* J Clin Psychopharmacol, 2000. **20**(3): p. 394-5.
106. Nierenberg, A.A., et al., *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): p. 1519-30; quiz 1665.
107. Kitaichi, Y., et al., *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): p. 236-45.
108. Redrobe, J.P. and M. Bourin, *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): p. 370-7.
109. Haddjeri, N., et al., *Increased tonic activation of rat forebrain 5-HT(1A) receptors by lithium addition to antidepressant treatments.* Neuropsychopharmacology, 2000. **22**(4): p. 346-56.
110. Muraki, I., et al., *Effect of subchronic lithium treatment on citalopram-induced increases in extracellular concentrations of serotonin in the medial prefrontal cortex.* J Neurochem, 2001. **76**(2): p. 490-7.
111. 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): p. 941-4.
112. Fisar, Z., J. Hroudova, and J. Raboch, *Inhibition of monoamine oxidase activity by antidepressants and mood stabilizers.* Neuro Endocrinol Lett, 2010. **31**(5): p. 645-56.
113. Reveley, M.A., et al., *Increased platelet monoamine oxidase activity in affective disorders.* Psychopharmacology (Berl), 1981. **73**(3): p. 257-60.

114. Shalbuyeva, N., T. Brustovetsky, and N. Brustovetsky, *Lithium desensitizes brain mitochondria to calcium, antagonizes permeability transition, and diminishes cytochrome C release.* J Biol Chem, 2007. **282**(25): p. 18057-68.
115. Haas, R., et al., *Inhibitory effects of sodium valproate on oxidative phosphorylation.* Neurology, 1981. **31**(11): p. 1473-6.
116. Hroudova, J. and Z. Fisar, *Activities of respiratory chain complexes and citrate synthase influenced by pharmacologically different antidepressants and mood stabilizers.* Neuro Endocrinol Lett, 2010. **31**(3): p. 336-42.
117. Correa, C., et al., *Effects of lithium and valproate on hippocampus citrate synthase activity in an animal model of mania.* Prog Neuropsychopharmacol Biol Psychiatry, 2007. **31**(4): p. 887-91.
118. Lai, J.S., et al., *Cytoprotection by lithium and valproate varies between cell types and cellular stresses.* Eur J Pharmacol, 2006. **539**(1-2): p. 18-26.
119. Bachmann, R.F., et al., *Common effects of lithium and valproate on mitochondrial functions: protection against methamphetamine-induced mitochondrial damage.* Int J Neuropsychopharmacol, 2009. **12**(6): p. 805-22.
120. Mai, L., R.S. Jope, and X. Li, *BDNF-mediated signal transduction is modulated by GSK3beta and mood stabilizing agents.* J Neurochem, 2002. **82**(1): p. 75-83.
121. Bown, C.D., J.F. Wang, and L.T. Young, *Attenuation of N-methyl-D-aspartate-mediated cytoplasmic vacuolization in primary rat hippocampal neurons by mood stabilizers.* Neuroscience, 2003. **117**(4): p. 949-55.
122. Berger, I., et al., *The effect of antiepileptic drugs on mitochondrial activity: a pilot study.* J Child Neurol, 2010. **25**(5): p. 541-5.
123. Chen, G., et al., *Attenuation of cyclic AMP production by carbamazepine.* J Neurochem, 1996. **67**(5): p. 2079-86.
124. Kim, Y.J., et al., *Lamotrigine inhibition of rotenone- or 1-methyl-4-phenylpyridinium-induced mitochondrial damage and cell death.* Brain Res Bull, 2007. **71**(6): p. 633-40.
125. Ponchaut, S., F. van Hoof, and K. Veitch, *In vitro effects of valproate and valproate metabolites on mitochondrial oxidations. Relevance of CoA sequestration to the observed inhibitions.* Biochem Pharmacol, 1992. **43**(11): p. 2435-42.
126. Luder, A.S., et al., *Inactivation of beef brain alpha-ketoglutarate dehydrogenase complex by valproic acid and valproic acid metabolites. Possible mechanism of anticonvulsant and toxic actions.* J Clin Invest, 1990. **86**(5): p. 1574-81.
127. Aires, C.C., et al., *Pyruvate uptake is inhibited by valproic acid and metabolites in mitochondrial membranes.* FEBS Lett, 2008. **582**(23-24): p. 3359-66.
128. Silva, M.F., et al., *Valproate inhibits the mitochondrial pyruvate-driven oxidative phosphorylation in vitro.* J Inherit Metab Dis, 1997. **20**(3): p. 397-400.
129. Valvassori, S.S., et al., *Effects of mood stabilizers on mitochondrial respiratory chain activity in brain of rats treated with d-amphetamine.* J Psychiatr Res, 2010. **44**(14): p. 903-9.
130. de Vasconcellos, A.P., et al., *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): p. 102-10.
131. Maurer, I.C., P. Schippel, and H.P. Volz, *Lithium-induced enhancement of mitochondrial oxidative phosphorylation in human brain tissue.* Bipolar Disord, 2009. **11**(5): p. 515-22.

132. King, T.D., G.N. Bijur, and R.S. Jope, *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): p. 106-14.
133. Santos, N.A., et al., *Aromatic antiepileptic drugs and mitochondrial toxicity: effects on mitochondria isolated from rat liver.* Toxicol In Vitro, 2008. **22**(5): p. 1143-52.
134. Costa, C., et al., *Electrophysiology and pharmacology of striatal neuronal dysfunction induced by mitochondrial complex I inhibition.* J Neurosci, 2008. **28**(32): p. 8040-52.
135. Fagervall, I. and S.B. Ross, *Inhibition of monoamine oxidase in monoaminergic neurones in the rat brain by irreversible inhibitors.* Biochem Pharmacol, 1986. **35**(8): p. 1381-7.
136. Smith, T.E., H. Weissbach, and S. Udenfriend, *STUDIES ON MONOAMINE OXIDASE: THE MECHANISM OF INHIBITION OF MONOAMINE OXIDASE BY IPRONIAZID.* Biochemistry, 1963. **2**: p. 746-51.
137. Fisar, Z. and J. Hroudova, *Intracellular signalling pathways and mood disorders.* Folia Biol (Praha), 2010. **56**(4): p. 135-48.
138. Fisar, Z., *Cannabinoids and monoamine neurotransmission with focus on monoamine oxidase.* Prog Neuropsychopharmacol Biol Psychiatry, 2012. **38**(1): p. 68-77.
139. Youdim, M.B. and Y.S. Bakhle, *Monoamine oxidase: isoforms and inhibitors in Parkinson's disease and depressive illness.* Br J Pharmacol, 2006. **147 Suppl 1**: p. S287-96.
140. Haefely, W., et al., *Biochemistry and pharmacology of moclobemide, a prototype RIMA.* Psychopharmacology (Berl), 1992. **106 Suppl**: p. S6-14.
141. Lecrubier, Y. and J.D. Guelfi, *Efficacy of reversible inhibitors of monoamine oxidase-A in various forms of depression.* Acta Psychiatr Scand Suppl, 1990. **360**: p. 18-23.
142. Lecrubier, Y., *Risk-benefit assessment of newer versus older monoamine oxidase (MAO) inhibitors.* Drug Saf, 1994. **10**(4): p. 292-300.
143. Iwersen, S. and A. Schmoldt, *Three suicide attempts with moclobemide.* J Toxicol Clin Toxicol, 1996. **34**(2): p. 223-5.
144. Lotufo-Neto, F., M. Trivedi, and M.E. Thase, *Meta-analysis of the reversible inhibitors of monoamine oxidase type A moclobemide and brofaromine for the treatment of depression.* Neuropsychopharmacology, 1999. **20**(3): p. 226-47.
145. Bonnet, U., *Moclobemide: therapeutic use and clinical studies.* CNS Drug Rev, 2003. **9**(1): p. 97-140.
146. Prasko, J., et al., *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): p. 473-81.
147. Jauch, R., et al., *Biotransformation of moclobemide in humans.* Acta Psychiatr Scand Suppl, 1990. **360**: p. 87-90.
148. Da Prada, M., et al., *Neurochemical profile of moclobemide, a short-acting and reversible inhibitor of monoamine oxidase type A.* J Pharmacol Exp Ther, 1989. **248**(1): p. 400-14.
149. Baker, G.B., et al., *Metabolism of monoamine oxidase inhibitors.* Cell Mol Neurobiol, 1999. **19**(3): p. 411-26.
150. Ramsay, R.R. and M.B. Gravestock, *Monoamine oxidases: to inhibit or not to inhibit.* Mini Rev Med Chem, 2003. **3**(2): p. 129-36.
151. Reyes, E. and J. Lisansky, *Effects of tricyclic antidepressants on platelet monoamine oxidase activity.* Clin Pharmacol Ther, 1984. **35**(4): p. 531-4.
152. Roth, J.A. and C.N. Gillis, *Inhibition of lung, liver and brain monoamine oxidase by imipramine and desipramine.* Biochem Pharmacol, 1974. **23**(6): p. 1138-40.

153. Edwards, D.J. and M.O. Burns, *Effects of tricyclic antidepressants upon human platelet monoamine oxidase.* Life Sci, 1974. **15**(12): p. 2045-58.
154. Sullivan, J.L., C. Dackis, and C. Stanfield, *In vivo inhibition of platelet MAO activity by tricyclic antidepressants.* Am J Psychiatry, 1977. **134**(2): p. 188-90.
155. Sullivan, J.L., et al., *Clinical correlates of tricyclic antidepressant-mediated inhibition of platelet monoamine oxidase.* Biol Psychiatry, 1978. **13**(3): p. 399-407.
156. Davidson, J., et al., *Platelet MAO inhibition following tricyclic antidepressant therapy.* Am J Psychiatry, 1978. **135**(5): p. 603-5.
157. Reveley, M.A., et al., *Absence of platelet monamine oxidase inhibition during amitriptyline or zimelidine treatment.* Br J Clin Pharmacol, 1979. **8**(4): p. 375-8.
158. Green, A.L., J.E. O'Grady, and M. Vass, *The effect of some tricyclic antidepressants on the inhibition of mouse brain monoamine oxidase in-vivo by phenelzine.* J Pharm Pharmacol, 1989. **41**(1): p. 50-1.
159. Honecker, H., et al., *Critical evaluation of measurement of platelet monoamine oxidase in man.* J Clin Chem Clin Biochem, 1976. **14**(9): p. 453-8.
160. Egashira, T., F. Takayama, and Y. Yamanaka, *The inhibition of monoamine oxidase activity by various antidepressants: differences found in various mammalian species.* Jpn J Pharmacol, 1999. **81**(1): p. 115-21.
161. Egashira, T., F. Takayama, and Y. Yamanaka, *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): p. 773-8.
162. Gnerre, C., et al., *Interaction of psychotropic drugs with monoamine oxidase in rat brain.* J Pharm Pharmacol, 2001. **53**(8): p. 1125-30.
163. Mukherjee, J. and Z.Y. Yang, *Monoamine oxidase A inhibition by fluoxetine: an in vitro and in vivo study.* Synapse, 1999. **31**(4): p. 285-9.
164. Mukherjee, J. and Z.Y. Yang, *Evaluation of monoamine oxidase B inhibition by fluoxetine (Prozac): an in vitro and in vivo study.* Eur J Pharmacol, 1997. **337**(1): p. 111-4.
165. Eckert, A., et al., *Mitochondrial dysfunction, apoptotic cell death, and Alzheimer's disease.* Biochem Pharmacol, 2003. **66**(8): p. 1627-34.
166. Hsieh, H.L. and C.M. Yang, *Role of redox signaling in neuroinflammation and neurodegenerative diseases.* Biomed Res Int, 2013. **2013**: p. 484613.
167. Anandatheerthavarada, H.K. and L. Devi, *Amyloid precursor protein and mitochondrial dysfunction in Alzheimer's disease.* Neuroscientist, 2007. **13**(6): p. 626-38.
168. Moreira, P.I., M.S. Santos, and C.R. Oliveira, *Alzheimer's disease: a lesson from mitochondrial dysfunction.* Antioxid Redox Signal, 2007. **9**(10): p. 1621-30.
169. Sherif, F., et al., *Brain gamma-aminobutyrate aminotransferase (GABA-T) and monoamine oxidase (MAO) in patients with Alzheimer's disease.* J Neural Transm Park Dis Dement Sect, 1992. **4**(3): p. 227-40.
170. Cai, Z., *Monoamine oxidase inhibitors: promising therapeutic agents for Alzheimer's disease (Review).* Mol Med Rep, 2014. **9**(5): p. 1533-41.
171. Naoi, M., et al., *Involvement of type A monoamine oxidase in neurodegeneration: regulation of mitochondrial signaling leading to cell death or neuroprotection.* J Neural Transm Suppl, 2006(71): p. 67-77.
172. Youdim, M.B., D. Edmondson, and K.F. Tipton, *The therapeutic potential of monoamine oxidase inhibitors.* Nat Rev Neurosci, 2006. **7**(4): p. 295-309.

173. Yankner, B.A., *Mechanisms of neuronal degeneration in Alzheimer's disease.* Neuron, 1996. **16**(5): p. 921-32.
174. Lane, R.M., S.G. Potkin, and A. Enz, *Targeting acetylcholinesterase and butyrylcholinesterase in dementia.* Int J Neuropsychopharmacol, 2006. **9**(1): p. 101-24.
175. Winslow, B.T., et al., *Treatment of Alzheimer disease.* Am Fam Physician, 2011. **83**(12): p. 1403-12.
176. Arendt, T., et al., *Changes in acetylcholinesterase and butyrylcholinesterase in Alzheimer's disease resemble embryonic development--a study of molecular forms.* Neurochem Int, 1992. **21**(3): p. 381-96.
177. Greig, N.H., D.K. Lahiri, and K. Sambamurti, *Butyrylcholinesterase: an important new target in Alzheimer's disease therapy.* Int Psychogeriatr, 2002. **14 Suppl 1**: p. 77-91.
178. Mesulam, M., et al., *Widely spread butyrylcholinesterase can hydrolyze acetylcholine in the normal and Alzheimer brain.* Neurobiol Dis, 2002. **9**(1): p. 88-93.
179. Lin, M.T. and M.F. Beal, *Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases.* Nature, 2006. **443**(7113): p. 787-95.
180. Sas, K., et al., *Mitochondria, metabolic disturbances, oxidative stress and the kynurenine system, with focus on neurodegenerative disorders.* J Neurol Sci, 2007. **257**(1-2): p. 221-39.
181. Emerit, J., M. Edeas, and F. Bricaire, *Neurodegenerative diseases and oxidative stress.* Biomed Pharmacother, 2004. **58**(1): p. 39-46.
182. Schapira, A.H., et al., *Mitochondrial complex I deficiency in Parkinson's disease.* J Neurochem, 1990. **54**(3): p. 823-7.
183. Parker, W.D., Jr., S.J. Boyson, and J.K. Parks, *Abnormalities of the electron transport chain in idiopathic Parkinson's disease.* Ann Neurol, 1989. **26**(6): p. 719-23.
184. Moran, M., et al., *Mitochondrial respiratory chain dysfunction: implications in neurodegeneration.* Free Radic Biol Med, 2012. **53**(3): p. 595-609.
185. Petrozzi, L., et al., *Mitochondria and neurodegeneration.* Biosci Rep, 2007. **27**(1-3): p. 87-104.
186. Gilgun-Sherki, Y., E. Melamed, and D. Offen, *Oxidative stress induced-neurodegenerative diseases: the need for antioxidants that penetrate the blood brain barrier.* Neuropharmacology, 2001. **40**(8): p. 959-75.
187. Rhein, V., et al., *Amyloid-beta leads to impaired cellular respiration, energy production and mitochondrial electron chain complex activities in human neuroblastoma cells.* Cell Mol Neurobiol, 2009. **29**(6-7): p. 1063-71.
188. van den Heuvel, L.P., J.A. Smeitink, and R.J. Rodenburg, *Biochemical examination of fibroblasts in the diagnosis and research of oxidative phosphorylation (OXPHOS) defects.* Mitochondrion, 2004. **4**(5-6): p. 395-401.
189. Murphy, M.P., *How mitochondria produce reactive oxygen species.* Biochem J, 2009. **417**(1): p. 1-13.
190. Hroudová, J. and Z. Fišar, *Control mechanisms in mitochondrial oxidative phosphorylation*. 2013: Neural Regeneration Research. p. 363-375.
191. Starkov, A.A., *The role of mitochondria in reactive oxygen species metabolism and signaling.* Ann N Y Acad Sci, 2008. **1147**: p. 37-52.
192. Schonfeld, P. and L. Wojtczak, *Fatty acids as modulators of the cellular production of reactive oxygen species.* Free Radic Biol Med, 2008. **45**(3): p. 231-41.
193. Boffoli, D., et al., *Decline with age of the respiratory chain activity in human skeletal muscle.* Biochim Biophys Acta, 1994. **1226**(1): p. 73-82.

194. Brieger, K., et al., *Reactive oxygen species: from health to disease.* Swiss Med Wkly, 2012. **142**: p. w13659.
195. Mattson, M.P., M. Gleichmann, and A. Cheng, *Mitochondria in neuroplasticity and neurological disorders.* Neuron, 2008. **60**(5): p. 748-66.
196. Aronis, A., et al., *Potentiation of Fas-mediated apoptosis by attenuated production of mitochondria-derived reactive oxygen species.* Cell Death Differ, 2003. **10**(3): p. 335-44.
197. Lu, B., *Mitochondrial dynamics and neurodegeneration.* Curr Neurol Neurosci Rep, 2009. **9**(3): p. 212-9.
198. Musatov, A. and N.C. Robinson, *Susceptibility of mitochondrial electron-transport complexes to oxidative damage. Focus on cytochrome c oxidase.* Free Radic Res, 2012. **46**(11): p. 1313-26.
199. Paradies, G., et al., *Reactive oxygen species affect mitochondrial electron transport complex I activity through oxidative cardiolipin damage.* Gene, 2002. **286**(1): p. 135-41.
200. Chaturvedi, R.K. and M. Flint Beal, *Mitochondrial diseases of the brain.* Free Radic Biol Med, 2013. **63**: p. 1-29.
201. Paradies, G., et al., *Functional role of cardiolipin in mitochondrial bioenergetics.* Biochim Biophys Acta, 2014. **1837**(4): p. 408-17.
202. Yin, H. and M. Zhu, *Free radical oxidation of cardiolipin: chemical mechanisms, detection and implication in apoptosis, mitochondrial dysfunction and human diseases.* Free Radic Res, 2012. **46**(8): p. 959-74.
203. Hashimoto, M., et al., *Role of protein aggregation in mitochondrial dysfunction and neurodegeneration in Alzheimer's and Parkinson's diseases.* Neuromolecular Med, 2003. **4**(1-2): p. 21-36.
204. Parker, W.D., Jr., C.M. Filley, and J.K. Parks, *Cytochrome oxidase deficiency in Alzheimer's disease.* Neurology, 1990. **40**(8): p. 1302-3.
205. Mutisya, E.M., A.C. Bowling, and M.F. Beal, *Cortical cytochrome oxidase activity is reduced in Alzheimer's disease.* J Neurochem, 1994. **63**(6): p. 2179-84.
206. Maurer, I., S. Zierz, and H.J. Moller, *A selective defect of cytochrome c oxidase is present in brain of Alzheimer disease patients.* Neurobiol Aging, 2000. **21**(3): p. 455-62.
207. Valla, J., et al., *Impaired platelet mitochondrial activity in Alzheimer's disease and mild cognitive impairment.* Mitochondrion, 2006. **6**(6): p. 323-30.
208. Candy, J.M., et al., *Pathological changes in the nucleus of Meynert in Alzheimer's and Parkinson's diseases.* J Neurol Sci, 1983. **59**(2): p. 277-89.
209. Fodero, L.R., et al., *Alpha7-nicotinic acetylcholine receptors mediate an Abeta(1-42)-induced increase in the level of acetylcholinesterase in primary cortical neurones.* J Neurochem, 2004. **88**(5): p. 1186-93.
210. Feldhaus, P., et al., *Evaluation of respiratory chain activity in lymphocytes of patients with Alzheimer disease.* Metab Brain Dis, 2011. **26**(3): p. 229-36.
211. Gibson, G.E., et al., *Cause and consequence: mitochondrial dysfunction initiates and propagates neuronal dysfunction, neuronal death and behavioral abnormalities in age-associated neurodegenerative diseases.* Biochim Biophys Acta, 2010. **1802**(1): p. 122-34.
212. Navarro, A. and A. Boveris, *The mitochondrial energy transduction system and the aging process.* Am J Physiol Cell Physiol, 2007. **292**(2): p. C670-86.
213. Kilbride, S.M., et al., *High-level inhibition of mitochondrial complexes III and IV is required to increase glutamate release from the nerve terminal.* Mol Neurodegener, 2011. **6**(1): p. 53.

214. Kilbride, S.M., et al., *Partial inhibition of complex I activity increases Ca-independent glutamate release rates from depolarized synaptosomes.* J Neurochem, 2008. **106**(2): p. 826-34.
215. Casademont, J., et al., *Cholinesterase inhibitor rivastigmine enhance the mitochondrial electron transport chain in lymphocytes of patients with Alzheimer's disease.* J Neurol Sci, 2003. **206**(1): p. 23-6.
216. Rhein, V., et al., *Amyloid-beta and tau synergistically impair the oxidative phosphorylation system in triple transgenic Alzheimer's disease mice.* Proc Natl Acad Sci U S A, 2009. **106**(47): p. 20057-62.
217. Bosetti, F., et al., *Cytochrome c oxidase and mitochondrial F1F0-ATPase (ATP synthase) activities in platelets and brain from patients with Alzheimer's disease.* Neurobiol Aging, 2002. **23**(3): p. 371-6.
218. Valla, J., J.D. Berndt, and F. Gonzalez-Lima, *Energy hypometabolism in posterior cingulate cortex of Alzheimer's patients: superficial laminar cytochrome oxidase associated with disease duration.* J Neurosci, 2001. **21**(13): p. 4923-30.
219. Parker, W.D., Jr., et al., *Electron transport chain defects in Alzheimer's disease brain.* Neurology, 1994. **44**(6): p. 1090-6.
220. Baloyannis, S.J., V. Costa, and D. Michmizos, *Mitochondrial alterations in Alzheimer's disease.* Am J Alzheimers Dis Other Demen, 2004. **19**(2): p. 89-93.
221. Kim, S.H., et al., *Decreased levels of complex III core protein 1 and complex V beta chain in brains from patients with Alzheimer's disease and Down syndrome.* Cell Mol Life Sci, 2000. **57**(12): p. 1810-6.
222. Kish, S.J., et al., *Brain cytochrome oxidase in Alzheimer's disease.* J Neurochem, 1992. **59**(2): p. 776-9.
223. Kim, S.H., et al., *The reduction of NADH ubiquinone oxidoreductase 24- and 75-kDa subunits in brains of patients with Down syndrome and Alzheimer's disease.* Life Sci, 2001. **68**(24): p. 2741-50.
224. Gan, X., et al., *Inhibition of ERK-DLP1 signaling and mitochondrial division alleviates mitochondrial dysfunction in Alzheimer's disease cybrid cell.* Biochim Biophys Acta, 2014. **1842**(2): p. 220-31.
225. Kushnareva, Y., A.N. Murphy, and A. Andreyev, *Complex I-mediated reactive oxygen species generation: modulation by cytochrome c and NAD(P)+ oxidation-reduction state.* Biochem J, 2002. **368**(Pt 2): p. 545-53.
226. Caspersen, C., et al., *Mitochondrial Abeta: a potential focal point for neuronal metabolic dysfunction in Alzheimer's disease.* Faseb j, 2005. **19**(14): p. 2040-1.
227. Cardoso, S.M., et al., *Cytochrome c oxidase is decreased in Alzheimer's disease platelets.* Neurobiol Aging, 2004. **25**(1): p. 105-10.
228. Kamat, P.K., et al., *Mitochondrial dysfunction: a crucial event in okadaic acid (ICV) induced memory impairment and apoptotic cell death in rat brain*, in *Pharmacol Biochem Behav*. 2011, 2011 Elsevier B.V: United States. p. 311-9.
229. Arias, E., et al., *Unequal neuroprotection afforded by the acetylcholinesterase inhibitors galantamine, donepezil, and rivastigmine in SH-SY5Y neuroblastoma cells: role of nicotinic receptors.* J Pharmacol Exp Ther, 2005. **315**(3): p. 1346-53.
230. Noh, M.Y., et al., *Neuroprotective effects of donepezil through inhibition of GSK-3 activity in amyloid-beta-induced neuronal cell death.* J Neurochem, 2009. **108**(5): p. 1116-25.

231. Takada, Y., et al., *Nicotinic acetylcholine receptor-mediated neuroprotection by donepezil against glutamate neurotoxicity in rat cortical neurons.* J Pharmacol Exp Ther, 2003. **306**(2): p. 772-7.
232. Ki, Y.S., et al., *Donepezil, a potent acetylcholinesterase inhibitor, induces caspase-dependent apoptosis in human promyelocytic leukemia HL-60 cells.* Biol Pharm Bull, 2010. **33**(6): p. 1054-9.
233. Ellis, J.M., *Cholinesterase inhibitors in the treatment of dementia.* J Am Osteopath Assoc, 2005. **105**(3): p. 145-58.
234. Jann, M.W., *Rivastigmine, a new-generation cholinesterase inhibitor for the treatment of Alzheimer's disease.* Pharmacotherapy, 2000. **20**(1): p. 1-12.
235. Parnetti, L., et al., *Cerebrospinal fluid levels of biomarkers and activity of acetylcholinesterase (AChE) and butyrylcholinesterase in AD patients before and after treatment with different AChE inhibitors.* Neurol Sci, 2002. **23 Suppl 2**: p. S95-6.
236. Amici, S., et al., *Cerebrospinal fluid acetylcholinesterase activity after long-term treatment with donepezil and rivastigmina.* Mech Ageing Dev, 2001. **122**(16): p. 2057-62.
237. Hellstrom-Lindahl, E., H. Moore, and A. Nordberg, *Increased levels of tau protein in SH-SY5Y cells after treatment with cholinesterase inhibitors and nicotinic agonists.* J Neurochem, 2000. **74**(2): p. 777-84.
238. Polinsky, R.J., *Clinical pharmacology of rivastigmine: a new-generation acetylcholinesterase inhibitor for the treatment of Alzheimer's disease.* Clin Ther, 1998. **20**(4): p. 634-47.
239. Bartorelli, L., et al., *Effects of switching from an AChE inhibitor to a dual AChE-BuChE inhibitor in patients with Alzheimer's disease.* Curr Med Res Opin, 2005. **21**(11): p. 1809-18.
240. Auriacombe, S., et al., *Efficacy and safety of rivastigmine in patients with Alzheimer's disease who failed to benefit from treatment with donepezil.* Curr Med Res Opin, 2002. **18**(3): p. 129-38.
241. Ballard, C.G., *Advances in the treatment of Alzheimer's disease: benefits of dual cholinesterase inhibition.* Eur Neurol, 2002. **47**(1): p. 64-70.
242. Bailey, J.A., et al., *Rivastigmine lowers Abeta and increases sAPPalpha levels, which parallel elevated synaptic markers and metabolic activity in degenerating primary rat neurons.* PLoS One, 2011. **6**(7): p. e21954.
243. Kumar, P. and A. Kumar, *Protective effect of rivastigmine against 3-nitropropionic acid-induced Huntington's disease like symptoms: possible behavioural, biochemical and cellular alterations.* Eur J Pharmacol, 2009. **615**(1-3): p. 91-101.
244. Casademont, J., et al., *Cholinesterase inhibitor rivastigmine enhance the mitochondrial electron transport chain in lymphocytes of patients with Alzheimer's disease*, in *J Neurol Sci*. 2003: Netherlands. p. 23-6.
245. Rogawski, M.A., *What is the rationale for new treatment strategies in Alzheimer's disease?* CNS Spectr, 2004. **9**(7 Suppl 5): p. 6-12.
246. Maelicke, A., *Allosteric modulation of nicotinic receptors as a treatment strategy for Alzheimer's disease.* Dement Geriatr Cogn Disord, 2000. **11 Suppl 1**: p. 11-8.
247. Arias, E., et al., *Galantamine prevents apoptosis induced by beta-amyloid and thapsigargin: involvement of nicotinic acetylcholine receptors.* Neuropharmacology, 2004. **46**(1): p. 103-14.

248. Liu, X., et al., *Protective effects of galantamine against Abeta-induced PC12 cell apoptosis by preventing mitochondrial dysfunction and endoplasmic reticulum stress.* Neurochem Int, 2010. **57**(5): p. 588-99.
249. Ezoulin, M.J., et al., *Differential effect of PMS777, a new type of acetylcholinesterase inhibitor, and galanthamine on oxidative injury induced in human neuroblastoma SK-N-SH cells.* Neurosci Lett, 2005. **389**(2): p. 61-5.
250. Kumar, A., A. Prakash, and D. Pahwa, *Galantamine potentiates the protective effect of rofecoxib and caffeic acid against intrahippocampal Kainic acid-induced cognitive dysfunction in rat.* Brain Res Bull, 2011. **85**(3-4): p. 158-68.
251. Lipton, S.A., *Paradigm shift in NMDA receptor antagonist drug development: molecular mechanism of uncompetitive inhibition by memantine in the treatment of Alzheimer's disease and other neurologic disorders.* J Alzheimers Dis, 2004. **6**(6 Suppl): p. S61-74.
252. Lipton, S.A., *The molecular basis of memantine action in Alzheimer's disease and other neurologic disorders: low-affinity, uncompetitive antagonism.* Curr Alzheimer Res, 2005. **2**(2): p. 155-65.
253. Kysenius, K., C.A. Brunello, and H.J. Huttunen, *Mitochondria and NMDA receptor-dependent toxicity of berberine sensitizes neurons to glutamate and rotenone injury*, in *PLoS One*. 2014: United States. p. e107129.
254. Tozzi, A., et al., *Memantine reduces neuronal dysfunctions triggered by in vitro ischemia and 3-nitropropionic acid.* Exp Neurol, 2007. **207**(2): p. 218-26.
255. Alley, G.M., et al., *Memantine lowers amyloid-beta peptide levels in neuronal cultures and in APP/PS1 transgenic mice.* J Neurosci Res, 2010. **88**(1): p. 143-54.
256. Arif, M., et al., *Effects of memantine on soluble Alphabeta(25-35)-induced changes in peptidergic and glial cells in Alzheimer's disease model rat brain regions.* Neuroscience, 2009. **164**(3): p. 1199-209.
257. Miguel-Hidalgo, J.J., et al., *Neuroprotection by memantine against neurodegeneration induced by beta-amyloid(1-40).* Brain Res, 2002. **958**(1): p. 210-21.
258. Song, M.S., et al., *Memantine protects rat cortical cultured neurons against beta-amyloid-induced toxicity by attenuating tau phosphorylation.* Eur J Neurosci, 2008. **28**(10): p. 1989-2002.
259. Wang, Z.F. and X.C. Tang, *Huperzine A protects C6 rat glioma cells against oxygen-glucose deprivation-induced injury.* FEBS Lett, 2007. **581**(4): p. 596-602.
260. Ashani, Y., J.O. Peggins, 3rd, and B.P. Doctor, *Mechanism of inhibition of cholinesterases by huperzine A*, in *Biochem Biophys Res Commun*. 1992: United States. p. 719-26.
261. Gordon, R.K., et al., *The NMDA receptor ion channel: a site for binding of Huperzine A*, in *J Appl Toxicol*. 2001, 2001 John Wiley & Sons, Ltd.: England. p. S47-51.
262. Zhang, H.Y., *New insights into huperzine A for the treatment of Alzheimer's disease.* Acta Pharmacol Sin, 2012. **33**(9): p. 1170-5.
263. Yang, L., et al., *Decreased accumulation of subcellular amyloid-beta with improved mitochondrial function mediates the neuroprotective effect of huperzine A*, in *J Alzheimers Dis*. 2012: Netherlands. p. 131-42.
264. Wang, C.Y., et al., *Huperzine A activates Wnt/beta-catenin signaling and enhances the nonamyloidogenic pathway in an Alzheimer transgenic mouse model.* Neuropsychopharmacology, 2011. **36**(5): p. 1073-89.

265. Wang, R., H. Yan, and X.C. Tang, *Progress in studies of huperzine A, a natural cholinesterase inhibitor from Chinese herbal medicine.* Acta Pharmacol Sin, 2006. **27**(1): p. 1-26.
266. Ye, C.Y., et al., *Donepezil attenuates Abeta-associated mitochondrial dysfunction and reduces mitochondrial Abeta accumulation in vivo and in vitro.* Neuropharmacology, 2015.
267. Korabecny, J., et al., *Synthesis and in vitro evaluation of N-alkyl-7-methoxytacrine hydrochlorides as potential cholinesterase inhibitors in Alzheimer disease.* Bioorg Med Chem Lett, 2010. **20**(20): p. 6093-5.
268. Soukup, O., et al., *A resurrection of 7-MEOTA: a comparison with tacrine.* Curr Alzheimer Res, 2013. **10**(8): p. 893-906.
269. Hroudova, J., et al., *In vitro effects of acetylcholinesterase inhibitors and reactivators on Complex I of electron transport chain.* Neuro Endocrinol Lett, 2011. **32**(3): p. 259-63.
270. Cavalli, A., et al., *Multi-target-directed ligands to combat neurodegenerative diseases.* J Med Chem, 2008. **51**(3): p. 347-72.
271. Lan, J.S., et al., *Design, synthesis and evaluation of novel tacrine-(beta-carboline) hybrids as multifunctional agents for the treatment of Alzheimer's disease.* Bioorg Med Chem, 2014. **22**(21): p. 6089-104.
272. Li, S.Y., et al., *Multifunctional tacrine-flavonoid hybrids with cholinergic, beta-amyloid-reducing, and metal chelating properties for the treatment of Alzheimer's disease.* Eur J Med Chem, 2013. **69**: p. 632-46.
273. Mao, F., et al., *Tacrine-propargylamine derivatives with improved acetylcholinesterase inhibitory activity and lower hepatotoxicity as a potential lead compound for the treatment of Alzheimer's disease.* J Enzyme Inhib Med Chem, 2015: p. 1-7.
274. Wang, Y., et al., *Novel multipotent phenylthiazole-tacrine hybrids for the inhibition of cholinesterase activity, beta-amyloid aggregation and Ca(2)(+) overload.* Bioorg Med Chem, 2012. **20**(21): p. 6513-22.
275. Xie, S.S., et al., *Multifunctional tacrine-trolox hybrids for the treatment of Alzheimer's disease with cholinergic, antioxidant, neuroprotective and hepatoprotective properties.* Eur J Med Chem, 2015. **93**: p. 42-50.
276. Xie, S.S., et al., *Design, synthesis and evaluation of novel tacrine-coumarin hybrids as multifunctional cholinesterase inhibitors against Alzheimer's disease.* Eur J Med Chem, 2013. **64**: p. 540-53.
277. Bautista-Aguilera, O.M., et al., *Design, synthesis, pharmacological evaluation, QSAR analysis, molecular modeling and ADMET of novel donepezil-indolyl hybrids as multipotent cholinesterase/monoamine oxidase inhibitors for the potential treatment of Alzheimer's disease.* Eur J Med Chem, 2014. **75**: p. 82-95.
278. Kitani, K., et al., *Why (--)deprenyl prolongs survivals of experimental animals: increase of anti-oxidant enzymes in brain and other body tissues as well as mobilization of various humoral factors may lead to systemic anti-aging effects*, in *Mech Ageing Dev*. 2002: Ireland. p. 1087-100.
279. Kiray, M., et al., *Deprenyl and the relationship between its effects on spatial memory, oxidant stress and hippocampal neurons in aged male rats*, in *Physiol Res*. 2006: Czech Republic. p. 205-12.
280. Sterling, J., et al., *Novel dual inhibitors of AChE and MAO derived from hydroxy aminoindan and phenethylamine as potential treatment for Alzheimer's disease*, in *J Med Chem*. 2002: United States. p. 5260-79.

281. Bolea, I., et al., *Synthesis, biological evaluation, and molecular modeling of donepezil and N-[(5-(benzyloxy)-1-methyl-1H-indol-2-yl)methyl]-N-methylprop-2-yn-1-amine hybrids as new multipotent cholinesterase/monoamine oxidase inhibitors for the treatment of Alzheimer's disease.* J Med Chem, 2011. **54**(24): p. 8251-70.
282. Lu, C., et al., *A novel series of tacrine-selegiline hybrids with cholinesterase and monoamine oxidase inhibition activities for the treatment of Alzheimer's disease.* Eur J Med Chem, 2013. **62**: p. 745-53.
283. Martinez, A. and A. Castro, *Novel cholinesterase inhibitors as future effective drugs for the treatment of Alzheimer's disease.* Expert Opin Investig Drugs, 2006. **15**(1): p. 1-12.
284. Maruyama, W., et al., *Anti-apoptotic action of anti-Alzheimer drug, TV3326 [(N-propargyl)-(3R)-aminoindan-5-yl]-ethyl methyl carbamate, a novel cholinesterase-monoamine oxidase inhibitor.* Neurosci Lett, 2003. **341**(3): p. 233-6.
285. Weinstock, M., et al., *TV3326, a novel neuroprotective drug with cholinesterase and monoamine oxidase inhibitory activities for the treatment of Alzheimer's disease.* J Neural Transm Suppl, 2000(60): p. 157-69.
286. Weinstock, M., et al., *Neuroprotective effects of novel cholinesterase inhibitors derived from rasagiline as potential anti-Alzheimer drugs.* Ann N Y Acad Sci, 2001. **939**: p. 148-61.
287. Weinstock, M., et al., *Effect of TV3326, a novel monoamine-oxidase cholinesterase inhibitor, in rat models of anxiety and depression.* Psychopharmacology (Berl), 2002. **160**(3): p. 318-24.
288. Camps, P., et al., *Tacrine-based dual binding site acetylcholinesterase inhibitors as potential disease-modifying anti-Alzheimer drug candidates.* Chem Biol Interact, 2010. **187**(1-3): p. 411-5.
289. Kozurkova M, H.S., Gazova Z, Paulikova H, Kristian P, *Neuroactive Multifunctional Tacrine Congeners with Cholinesterase, Anti-Amyloid Aggregation and Neuroprotective Properties.* Pharmaceuticals, 2011. **4**(2): p. 382-418.
290. Wang, L., et al., *Donepezil + propargylamine + 8-hydroxyquinoline hybrids as new multifunctional metal-chelators, ChE and MAO inhibitors for the potential treatment of Alzheimer's disease.* Eur J Med Chem, 2014. **80**: p. 543-61.
291. Fang, F. and G.T. Liu, *Protective effects of compound FLZ, a novel synthetic analogue of squamosamide, on beta-amyloid-induced rat brain mitochondrial dysfunction in vitro.* Acta Pharmacol Sin, 2009. **30**(5): p. 522-9.
292. Ye, X., et al., *FLZ inhibited gamma-secretase selectively and decreased Abeta mitochondrial production in APP-SH-SY5Y cells.* Naunyn Schmiedebergs Arch Pharmacol, 2014. **387**(1): p. 75-85.
293. Bao, X.Q., et al., *FLZ alleviates the memory deficits in transgenic mouse model of Alzheimer's disease via decreasing beta-amyloid production and tau hyperphosphorylation.* PLoS One, 2013. **8**(11): p. e78033.
294. Muirhead, K.E., et al., *The consequences of mitochondrial amyloid beta-peptide in Alzheimer's disease.* Biochem J, 2010. **426**(3): p. 255-70.
295. Valasani, K.R., et al., *Identification of human ABAD inhibitors for rescuing Abeta-mediated mitochondrial dysfunction.* Curr Alzheimer Res, 2014. **11**(2): p. 128-36.
296. Lustbader, J.W., et al., *ABAD directly links Abeta to mitochondrial toxicity in Alzheimer's disease.* Science, 2004. **304**(5669): p. 448-52.
297. Lim, Y.A., et al., *Inhibition of the mitochondrial enzyme ABAD restores the amyloid-beta-mediated deregulation of estradiol.* PLoS One, 2011. **6**(12): p. e28887.

298. Valasani, K.R., et al., *Structure-based design and synthesis of benzothiazole phosphonate analogues with inhibitors of human ABAD-Abeta for treatment of Alzheimer's disease.* Chem Biol Drug Des, 2013. **81**(2): p. 238-49.
299. Rao, V.K., E.A. Carlson, and S.S. Yan, *Mitochondrial permeability transition pore is a potential drug target for neurodegeneration.* Biochim Biophys Acta, 2014. **1842**(8): p. 1267-72.
300. Du, H., et al., *Cyclophilin D deficiency attenuates mitochondrial and neuronal perturbation and ameliorates learning and memory in Alzheimer's disease.* Nat Med, 2008. **14**(10): p. 1097-105.
301. Elkamhawy, A., et al., *Novel quinazoline-urea analogues as modulators for Abeta-induced mitochondrial dysfunction: design, synthesis, and molecular docking study.* Eur J Med Chem, 2014. **84**: p. 466-75.
302. Jan, K., et al., *Comparison of Novel Tacrine and 7-MEOTA Derivatives with Aromatic and Alicyclic Residues: Synthesis, Biological Evaluation and Docking Studies.* Letters in Organic Chemistry, 2013. **10**(4): p. 291-297.
303. Korabecny, J., et al., *Synthesis and in vitro evaluation of N-(Bromobut-3-en-2-yl)-7-methoxy-1,2,3,4-tetrahydroacridin-9-amine as a cholinesterase inhibitor with regard to Alzheimer's disease treatment.* Molecules, 2010. **15**(12): p. 8804-12.
304. Korabecny, J., et al., *Synthesis and in vitro evaluation of 7-methoxy-N-(pent-4-enyl)-1,2,3,4-tetrahydroacridin-9-amine-new tacrine derivate with cholinergic properties.* Bioorg Med Chem Lett, 2011. **21**(21): p. 6563-6.
305. Sakurada, K., et al., *Effects of oximes on mitochondrial oxidase activity.* Toxicol Lett, 2009. **189**(2): p. 110-4.
306. Suzuki, O., H. Seno, and T. Kumazawa, *In vitro inhibition of human platelet monoamine oxidase by phenothiazine derivatives.* Life Sci, 1988. **42**(21): p. 2131-6.
307. Fisar, Z., *Inhibition of monoamine oxidase activity by cannabinoids.* Naunyn Schmiedebergs Arch Pharmacol, 2010. **381**(6): p. 563-72.
308. Overall, J.E. and D.R. Gorham, *The brief psychiatric rating scale.* Psychol. Rep, 1962. **10**: p. 799-812.
309. Guy, W., *"Clinical Global Impressions (CGI) Scale, Modified". In Rush, John A.; Task Force for the Handbook of Psychiatric Measures. Handbook of Psychiatric Measures (1st ed.).* 2000: Washington D.C., American Psychiatric Association.
310. Lukasiewicz, M., et al., *Young Mania Rating Scale: how to interpret the numbers? Determination of a severity threshold and of the minimal clinically significant difference in the EMBLEM cohort.* Int J Methods Psychiatr Res, 2013. **22**(1): p. 46-58.
311. Bridges, H.R., J.A. Birrell, and J. Hirst, *The mitochondrial-encoded subunits of respiratory complex I (NADH:ubiquinone oxidoreductase): identifying residues important in mechanism and disease.* Biochem Soc Trans, 2011. **39**(3): p. 799-806.
312. Brown, M.D., et al., *Mitochondrial DNA sequence analysis of four Alzheimer's and Parkinson's disease patients.* Am J Med Genet, 1996. **61**(3): p. 283-9.
313. Iommarini, L., et al., *Complex I impairment in mitochondrial diseases and cancer: parallel roads leading to different outcomes.* Int J Biochem Cell Biol, 2013. **45**(1): p. 47-63.
314. McKenzie, M., et al., *Capture of somatic mtDNA point mutations with severe effects on oxidative phosphorylation in synaptosome cybrid clones from human brain.* Hum Mutat, 2014. **35**(12): p. 1476-84.

315. Telford, J.E., S.M. Kilbride, and G.P. Davey, *Complex I is rate-limiting for oxygen consumption in the nerve terminal.* J Biol Chem, 2009. **284**(14): p. 9109-14.
316. Pathak, R.U. and G.P. Davey, *Complex I and energy thresholds in the brain.* Biochim Biophys Acta, 2008. **1777**(7-8): p. 777-82.
317. Davey, G.P., S. Peuchen, and J.B. Clark, *Energy thresholds in brain mitochondria. Potential involvement in neurodegeneration.* J Biol Chem, 1998. **273**(21): p. 12753-7.
318. Wong-Riley, M.T., *Cytochrome oxidase: an endogenous metabolic marker for neuronal activity.* Trends Neurosci, 1989. **12**(3): p. 94-101.
319. Wang, J.F., *Defects of mitochondrial electron transport chain in bipolar disorder: implications for mood-stabilizing treatment.* Can J Psychiatry, 2007. **52**(12): p. 753-62.
320. Calabrese, V., et al., *Cellular stress responses, the hormesis paradigm, and vitagenes: novel targets for therapeutic intervention in neurodegenerative disorders.* Antioxid Redox Signal, 2010. **13**(11): p. 1763-811.
321. Mattiasson, G., *Analysis of mitochondrial generation and release of reactive oxygen species.* Cytometry A, 2004. **62**(2): p. 89-96.
322. Niklas, J., F. Noor, and E. Heinzle, *Effects of drugs in subtoxic concentrations on the metabolic fluxes in human hepatoma cell line Hep G2.* Toxicol Appl Pharmacol, 2009. **240**(3): p. 327-36.
323. Ezoulin, M.J., et al., *A new acetylcholinesterase inhibitor with anti-PAF activity modulates oxidative stress and pro-inflammatory mediators release in stimulated RAW 264.7 macrophage cells. Comparison with tacrine.* Int Immunopharmacol, 2007. **7**(13): p. 1685-94.
324. Ezoulin, M.J., et al., *Study of PMS777, a new type of acetylcholinesterase inhibitor, in human HepG2 cells. Comparison with tacrine and galanthamine on oxidative stress and mitochondrial impairment.* Toxicol In Vitro, 2006. **20**(6): p. 824-31.
325. Fowler, C.J. and K.F. Tipton, *Concentration dependence of the oxidation of tyramine by the two forms of rat liver mitochondrial monoamine oxidase.* Biochem Pharmacol, 1981. **30**(24): p. 3329-32.
326. Green, A.L. and H.A. McGachy, *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): p. 392-4.
327. Huang, R.H. and R. Faulkner, *The role of phospholipid in the multiple functional forms of brain monoamine oxidase.* J Biol Chem, 1981. **256**(17): p. 9211-5.
328. Fowler, P.W., et al., *Monotopic enzymes and lipid bilayers: a comparative study.* Biochemistry, 2007. **46**(11): p. 3108-15.
329. Muriel, P. and J.M. Perez-Rojas, *Nitric oxide inhibits mitochondrial monoamine oxidase activity and decreases outer mitochondria membrane fluidity.* Comp Biochem Physiol C Toxicol Pharmacol, 2003. **136**(3): p. 191-7.
330. Fisar, Z., et al., *Imipramine distribution among red blood cells, plasma and brain tissue.* Gen Physiol Biophys, 1996. **15**(1): p. 51-64.
331. Fisar, Z., et al., *Distribution of antidepressants between plasma and red blood cells.* Neuro Endocrinol Lett, 2006. **27**(3): p. 307-13.
332. Karson, C.N., et al., *Human brain fluoxetine concentrations.* J Neuropsychiatry Clin Neurosci, 1993. **5**(3): p. 322-9.
333. Caccia, S., et al., *Influence of dose and route of administration on the kinetics of fluoxetine and its metabolite norfluoxetine in the rat.* Psychopharmacology (Berl), 1990. **100**(4): p. 509-14.

334. Shadurskaia, S.K., et al., *[Neuromediator mechanisms of the effect of the antihistamine agent dimebone on the brain]*. Biull Eksp Biol Med, 1986. **101**(6): p. 700-2.
335. Stancheva, S.L. and L.G. Alova, *[Effect of centrophenoxine, piracetam and aniracetam on the monoamine oxidase activity in different brain structures of rats]*. Farmakol Toksikol, 1988. **51**(3): p. 16-8.
336. Voicu, V.A., et al., *Pharmacokinetics and pharmacodynamics of some oximes and associated therapeutic consequences: a critical review.* J Appl Toxicol, 2010. **30**(8): p. 719-29.
337. Bajgar, J., et al., *Treatment of organophosphate intoxication using cholinesterase reactivators: facts and fiction.* Mini Rev Med Chem, 2007. **7**(5): p. 461-6.
338. Gubert, C., et al., *Mitochondrial activity and oxidative stress markers in peripheral blood mononuclear cells of patients with bipolar disorder, schizophrenia, and healthy subjects.* J Psychiatr Res, 2013. **47**(10): p. 1396-402.
339. Fisar, Z., et al., *Mitochondrial Respiration in the Platelets of Patients with Alzheimer's Disease.* Curr Alzheimer Res, 2016. **13**(8): p. 930-41.
340. Bohm, M., et al., *Activities of respiratory chain complexes in isolated platelets in females with anorexia nervosa.* Int J Eat Disord, 2007. **40**(7): p. 659-63.
341. Ben-Shachar, D., *The interplay between mitochondrial complex I, dopamine and Sp1 in schizophrenia.* J Neural Transm (Vienna), 2009. **116**(11): p. 1383-96.
342. Hroudova, J. and Z. Fisar, *Connectivity between mitochondrial functions and psychiatric disorders.* Psychiatry Clin Neurosci, 2011. **65**(2): p. 130-41.