Charles University in Prague

First Faculty of Medicine

Doctoral Thesis Summary

(Autoreferát disertační práce)

FIRST FACULTY
OF MEDICINE
Charles University

In vitro effects of selected psychopharmaca on energy metabolism

Mgr. Matej Ľupták

Prague, 2023

Doctoral Degree Study Programs in Biomedicine

Charles University in Prague and The Academy of Sciences of the Czech Republic

Program: Pharmacology and Toxicology

Board Chair: prof. MUDr. Ondřej Slanař, Ph.D.

Institute: Institute of Pharmacology

Supervisor: doc. PharmDr. Jana Rečková Hroudová, Ph.D.

The thesis will be available for inspection at the Dean's Office: Department of Science and Research and International Relations of the First Faculty of Medicine, Charles University in Prague at least five days before the defense.

Contents

Abbreviations

ETC	electron transport chain
MAO	monoamine oxidase
OXPHOS	oxidative phosphorylation
ROS	reactive oxygen species

This research was supported by the Charles University Grant Agency (grant number 34119), by the Charles University, Prague, (project Cooperatio, research area Neurosciences), by grant project SVV 260 523, and by the project MH CZ – DRO VFN64165.

Abstrakt

Mitochondriální dysfunkce je klíčovým faktorem v patofyziologií psychiatrických onemocnění a může sloužit jako modulátor a prediktor terapeutické odpovědi. Porozumění účinkům antipsychotik a antidepresiv na mitochondrie je esenciální pro propojení jejich terapeutických a nežádoucích účinků s mitochondriálními funkcemi.

Ve studii byly použity jako *in vitro* model mitochondrie izolované z prasečích mozků. Hodnotili jsme vliv vybraných antipsychotik (aripiprazol, brexpiprazol, haloperidol, chlorpromazin, kariprazin, klozapin, levomepromazin, loxapin, lurasidon, olanzapin, quetiapin, risperidon, ziprasidon a zotepin) a antidepresiv (agomelatin, bupropion, escitalopram, fluvoxamin, ketamin, paroxetin, sertralin a vortioxetin) na mitochondriální funkce. Byly měřeny aktivity komplexů elektronového transportního řetězce I, II+III a IV, monoaminooxidázy (MAO), také mitochondriální respirace, produkce ATP a tvorba peroxidu vodíku.

Většina testovaných antipsychotik a všechna antidepresiva (kromě bupropionu u komplexu II+III) signifikantně inhibovala aktivitu komplexů elektronového transportního řetězce. Některá antipsychotika a vortioxetin výrazně inhibovala mitochondriální respiraci a byly u nich pozorovány statisticky významné změny v produkci ATP. Všechna testovaná antipsychotika, agomelatin a vortioxetin (50 μM) významně zvýšili produkci peroxidu vodíku. Brexpiprazol, loxapin a všechna testovaná antidepresiva účinkovaly jako částečné anebo plné inhibitory MAO A a/anebo B. Výsledky naznačují, že dlouhodobá inhibice oxidativní fosforylace může vést k nedostatku ATP v neuronech a jejich poškození při vysoké koncentraci psychofarmak. Mitochondriální účinky psychofarmak jsou pravděpodobně spojeny s nežádoucími účinky. Aktivace kompenzačních mechanismů a inhibice MAO ale mohou hrát roli v jejich terapeutických účincích.

Abstract

Mitochondrial dysfunction is a key factor in the psychiatric disorders pathophysiology and may modulate and predict therapeutic response. Understanding how antipsychotics and antidepressants affect mitochondria is essential to relate their therapeutic and adverse effects to mitochondrial function.

Mitochondria isolated from pig brain were used as an *in vitro* model. We evaluated the effects of selected antipsychotics (aripiprazole, brexpiprazole, haloperidol, chlorpromazine, cariprazine, clozapine, levomepromazine, loxapine, lurasidone, olanzapine, quetiapine, risperidone, ziprasidone, and zotepine) and antidepressants (agomelatine, bupropion, escitalopram, fluvoxamine, ketamine, paroxetine, sertraline, and vortioxetine) on mitochondrial functions. The activities of electron transport chain (ETC) complexes I, II+III, and IV, monoamine oxidase (MAO), and mitochondrial respiration, ATP production, and hydrogen peroxide formation were measured.

Most of the tested antipsychotics and all antidepressants (except bupropion for complex II+III) significantly inhibited the activity of the electron transport chain complexes. Several antipsychotics and vortioxetine significantly inhibited mitochondrial respiration, and statistically significant changes in ATP production were observed for them. All tested antipsychotics, agomelatine and vortioxetine (50 μM) significantly increased hydrogen peroxide production. Brexpiprazole, loxapine and all tested antidepressants acted as partial or full inhibitors of MAO A and/or B. The results suggest that prolonged inhibition of oxidative phosphorylation at high concentrations of psychotropic drugs may lead to ATP deficiency in neurons and their damage. The mitochondrial effects of psychotropic drugs are likely to be associated with adverse effects, while compensatory mechanisms and MAO inhibition may play a role in their therapeutic effects.

1. Introduction

Although major depressive disorder and schizophrenia are common psychiatric disorders, their pathophysiology on molecular level remains unclear (Montes, Ferrando et al. 2004, Fišar 2023). Currently research focuses more on the molecular and cellular mechanisms, including alterations in neuroplasticity, inflammatory and immune responses, oxidative stress and antioxidant capacity, and mitochondrial functions (Maes, Fišar et al. 2012). Several different pathophysiological hypotheses have been proposed, including the mitochondrial hypothesis.

The primary function of mitochondria is to generate ATP through oxidative phosphorylation (OXPHOS) using electron transport chain (ETC) complexes. While alterations in mitochondrial function can negatively impact brain energy consumption and increase susceptibility to psychiatric disorders, mitochondria have been implicated in the pathogenesis of psychiatric disorders (Hroudová and Fišar 2011, Filiou and Sandi 2019). Dysfunction in mitochondrial activity can have profound effects on neuroplasticity, a fundamental process that underlies the brain's ability to adapt and change and contributes to the development and maintenance of depressive and aberrant behavior (Devine and Kittler 2018, Ben-Shachar 2020). A wide range of mitochondrial dysfunction has been observed in patients with psychiatric disorders. Polymorphisms and mutations in mitochondrial and nuclear DNA lead to dysregulation of Ca^{2+}, abnormal cellular energy and metabolism, decreased pH and ATP formation, impaired function of respiratory complexes and enzymes, and increased levels of reactive oxygen species

(ROS). Postmortem examination of the brains of patients also revealed anatomical and neuroanatomical abnormalities (Jou, Chiu et al. 2009, Wood, Yücel et al. 2009, Hroudová and Fišar 2011, Manji, Kato et al. 2012, Toker and Agam 2015, van Rensburg, Lindeque et al. 2022, Fišar 2023).

2. Hypothesis and aims of the study

Classical pathophysiological hypotheses of depression (monoamine hypothesis) and schizophrenia (dopamine hypothesis) are well established, but cannot fully explain all aspects of the disease (Howes, McCutcheon et al. 2015, Allen, Romay-Tallon et al. 2018). We hypothesize that currently used antipsychotics and antidepressants exert effects on mitochondrial function, specifically on mitochondrial respiratory efficiency, ATP production, ROS generation, maintenance of calcium homeostasis, as well as apoptosis and neurodevelopment.

Based on this hypothesis, we investigated the *in vitro* effects of selected psychotropic drugs on cellular metabolism using an established model of a purified mitochondrial fraction isolated from pig cerebral cortex. We evaluated mitochondrial respiration, the activity of ETC complexes I, II+II, and IV, citric acid cycle enzymes (citrate synthase, malate dehydrogenase), and MAO-A and MAO-B, ATP levels and kinetics, and ROS formation.

The primary aim of this study was to investigate the *in vitro* effects of selected antipsychotics and antidepressants on cellular energy metabolism and mitochondrial functions. By studying these effects, we aim to elucidate the changes in mitochondrial parameters induced by the evaluated drugs and

to establish potential associations with their beneficial or adverse drug effects.

3. Materials and methods

Radiochemical, spectrophotometric, fluorescence methods, and high-resolution respirometry were used. The effects of pharmacologically different antipsychotics and antidepressants on mitochondrial function were measured as drug-induced changes in the activities of mitochondrial enzymes, especially both MAO isoforms (MAO-A, MAO-B) and enzymes of the citric acid cycle and ETC complexes. Oxygen consumption and its sensitivity to substrates, uncouplers, and inhibitors were also measured to indicate mitochondrial phosphorylation capacity. ATP content and kinetics and ROS generation were evaluated. The concentration of selected antipsychotics and antidepressants ranged from 0.1-1000 μM.

1.1. Isolation of animal mitochondria

Pig brains were obtained from a slaughterhouse and mitochondria were extracted from the cerebral cortex, purified by centrifugation on a sucrose gradient, and the protein concentration was determined by the Lowry method (Lowry, Rosebrough et al. 1951, Fišar and Hroudová 2016). The freshly prepared mitochondria were stored on ice and used for high-resolution respirometry and assessment of ATP and hydrogen peroxide production. The activity of ETC complexes, citrate synthase, malate dehydrogenase, and MAO was assessed using cryopreserved mitochondria stored at -70 °C.

1.2. Mitochondrial enzyme activities

The tested drugs were incubated with ultrasonicated mitochondria for 30 minutes at 30 °C. A drug-free control was included in each measurement. The activities of ETC complexes (I, II+III, and IV), citrate synthase, and malate dehydrogenase were measured spectrophotometrically, using the GENESYS 180 UV-Vis spectrophotometer ((Thermo Fisher Scientific) (Barrie Kitto 1969, Srere 1969, Rustin, Chretien et al. 1994, Trounce, Kim et al. 1996, Folbergrová, Jesina et al. 2010).

The enzymatic activity of MAO was assessed using radiolabeled substrates [^{3}H]serotonin for MAO-A and [^{14}C]PEA for MAO-B). Sample radioactivity was quantified by liquid scintillation counting (Egashira, Takayama et al. 1999, Fisar 2010).

1.3. Mitochondrial respiration

Mitochondrial oxygen consumption rate was determined by high-resolution respirometry using Oxygraph-2k (Oroboros Instruments Corp). Complex I-linked respiration was measured with malate and pyruvate, and complex II-linked respiration was measured with succinate as substrate (Sjövall, Morota et al. 2010, Hroudová and Fišar 2012). Four simultaneous titrations were performed with DMSO as a control.

1.4. ATP content and kinetics

The ATP Bioluminescence Assay Kit CLS II was used to quantify the ATP content and to assess ATP kinetics as luminescence using FluoroMax-3 (Jobin Yvon) (Tonkonogi and Sahlin 1997, Manfredi, Spinazzola et al. 2001).

1.5. Drug effect on ROS generation

The Amplex Red Hydrogen Peroxide/Peroxidase Assay Kit was used to determine the formation of hydrogen peroxide as luminescence. (Liu, Fiskum et al. 2002).

1.6. Data analysis

STATISTICA 12 software was used for data analysis. A one-sample *t*-test was performed, and the data were presented as mean ± standard deviation or mean ± standard error of the mean. Correlations between data were examined using a correlation matrix, with Pearson's correlation coefficient presented.

2. Results

2.1. Drug effects on the activity of mitochondrial enzymes

Several tested antipsychotics significantly decreased the activity of complex I. The inhibitory potential of the antipsychotics is as follows: chlorpromazine > haloperidol > cariprazine > zotepine > brexpiprazole > lurasidone > aripiprazole > loxapine > quetiapine > risperidone > clozapine. All tested antidepressants showed significant inhibitory effects on complex I and are listed from the most potent to the least potent: vortioxetine > sertraline > paroxetine > fluvoxamine > escitalopram > trazodone > agomelatine > bupropion > ketamine.

Significant inhibition of complex II+III activity was measured for antipsychotics loxapine, zotepine, lurasidone, aripiprazole, brexpiprazole, cariprazine, quetiapine, and risperidone. All tested antidepressants, except bupropion, significantly inhibited complex II+III activity, they are listed in descending order of inhibitory potency: vortioxetine >

sertraline > paroxetine > trazodone > ketamine ~ agomelatine ~ escitalopram ~ fluvoxamine.

Significant inhibitory activity against complex IV was observed with the following antipsychotics: zotepine, chlorpromazine, loxapine (10-50 μM), lurasidone, and levomepromazine. Brexpiprazole (50-100 μM), loxapine (100 μM), and quetiapine significantly increased complex IV activity. Inhibitory potency of antidepressants against complex IV decreased in the following order: ketamine, vortioxetine, agomelatine, trazodone, escitalopram, sertraline, paroxetine, bupropion, and fluvoxamine.

Antipsychotics brexpiprazole, cariprazine and loxapine significantly inhibited MAO-A activity and acted as partial inhibitors of MAO-A. All tested antidepressants significantly inhibited MAO-A activity, with escitalopram, fluvoxamine and paroxetine acting as full MAO-A inhibitors.

MAO-B was inhibited by all tested antipsychotics, with brexpiprazole and loxapine being partial MAO-B inhibitors.
All tested antidepressants significantly decreased MAO-B activity, with escitalopram and paroxetine being full MAO-B inhibitors.

2.2. Drug effects on mitochondrial respiration

Several tested antipsychotics significantly inhibited complex I-linked respiration. Cariprazine, aripiprazole, zotepine, haloperidol, quetiapine, and risperidone were identified as full inhibitors of complex I-linked respiration.
Vortioxetine was the only antidepressant that was a full inhibitor of complex I-linked respiration.

Lurasidone, zotepine, quetiapine, and clozapine were full inhibitors of complex II-linked respiration. Bupropion was the

only tested antidepressant with inhibitory properties against complex II-linked respiration acting as a partial inhibitor.

2.3. Drug effect on ATP formation

Significant decreases in ATP formation were observed with antipsychotics brexpiprazole, cariprazine, loxapine, and lurasidone. Several antidepressants significantly affected ATP content and kinetics.

2.4. Drug effect on ROS generation

All tested antipsychotics significantly increased hydrogen peroxide production at various concentrations, with loxapine being the most potent stimulator. Antidepressants agomelatine (10 μM) and vortioxetine (50 μM) were found to stimulate hydrogen peroxide production. On the other hand, vortioxetine (100 μM) significantly decreased hydrogen peroxide production.

3. Discussion

Drug-induced changes in mitochondrial function, energy cell metabolism, MAO-A and MAO-B activities were evaluated *in vitro* in isolated pig brain mitochondria using antipsychotics and antidepressants with different chemical structures and pharmacological mechanisms of action. The evaluation of mitochondrial effects of psychiatric drugs is crucial for understanding the pathophysiology of psychiatric disorders, elucidating the mechanisms behind therapeutic and adverse effects of psychotropic drugs, and identifying potential mitochondrial targets for new drugs. An isolated purified mitochondrial fraction from pig cerebral cortex was used as an *in vitro* model. It was previously established and evaluated for

its suitability to study the effects of drugs on mitochondrial functions (Fišar and Hroudová 2016).

Antipsychotics: Most tested antipsychotics significantly inhibited the activity of ETC complexes. Complex I, which plays a critical role in cellular energy metabolism and oxygen consumption, is the most sensitive component of OXPHOS to inhibition. Complex I inhibition can lead to disruption of energy metabolism, increased production of ROS, and a shift in ATP production from mitochondria to glycolysis and subsequent changes in neuronal activity (Pathak and Davey 2008, Hroudová and Fišar 2012). Although complex II is not directly involved in generating the proton motive force required for ATP production (Grimm 2013), it can still affect OXPHOS.

All tested antipsychotics showed a decrease in ATP production at concentrations of 100 μM, which may affect ATP-dependent cellular processes and neuronal functions, given the importance of ATP in physiological neuronal function, as a coenzyme, and as a signaling molecule (Filiou and Sandi 2019, Chen, Park et al. 2019).

All tested antipsychotics except lurasidone showed partial inhibition of MAO-A activity. This suggests that MAO-A inhibition may contribute to the antidepressant properties in addition to the more important serotonin 5-HT receptor partial agonism/antagonism, which is probably responsible for most of the antidepressant effects (Corponi, Fabbri et al. 2019, Fasipe 2019). Brexpiprazole and loxapine showed partial inhibition of MAO-B, which may have potential beneficial effects in the treatment of neurodegenerative disorders.

Antipsychotics have been associated with adverse effects related to mitochondrial dysfunction. Complex I inhibition has

been associated with extrapyramidal symptoms and QTc interval prolongation, and second-generation antipsychotics have also been associated with an increased risk of cardiovascular events and metabolic syndrome, which may be related to alterations in mitochondrial homeostasis (Glassman and Bigger 2001, Del Campo, Bustos et al. 2018). While antipsychotics can inhibit ETC complexes, their molecular structures alone do not determine their mitochondrial effects. The complexity of respiratory chain structures and the allosteric regulation of different components within the ETC contribute to the cumulative effects of antipsychotics on mitochondrial respiration. Nevertheless, some antipsychotics may have additive antidepressant or neuroprotective effects, or induce adaptive processes that compensate for mitochondrial toxicity, highlighting the complex balance between therapeutic and side effects of antipsychotics.

Antidepressants: All tested antidepressants significantly inhibited all ETC complexes activity and complex I-linked respiration, except bupropion and ketamine, which showed very weak or no inhibition of complex I and II+III activity and no inhibitory activity against complex I-linked respiration, confirming a strong but not trivial correlation between ETC complexes activity and complex I-linked respiration. Selective serotonin reuptake inhibitors and vortioxetine showed more potent complex I inhibition compared to other antidepressants tested. However, complex II-linked respiration remained unaffected, indicating that OXPHOS can still perform respiration through complex II. These results are consistent with our previous data with tricyclic antidepressants, selective serotonin reuptake inhibitors and ketamine, which showed significant inhibition of mitochondrial respiration and ETC

complexes activity (Hroudova and Fisar 2010, Hroudová and Fišar 2012).

We have shown that different antidepressants have different effects on mitochondrial ATP production and kinetics. Considering strong drug-induced inhibition of complexes I and IV activity, changes in ATP content and kinetics were smaller, suggesting that ATP production is less sensitive to antidepressant treatment compared to complex I activity. These findings highlight the complexity of mitochondrial ATP production, which cannot be determined by isolated ETC complexes activity or oxygen consumption rate alone.

No consistent trend of increased hydrogen peroxide production was observed with increasing concentrations of the tested antidepressants. ROS in mitochondria can act as signaling molecules, triggering compensatory mechanisms to restore redox balance, and modulating various cellular processes, including neuronal plasticity and neurotransmitter production (Speijer 2019). It is challenging to determine the consequences of antidepressant-induced changes in ROS levels due to their dual role as signaling molecules and contributors to oxidative damage.

All tested antidepressants showed either full or partial inhibition of MAO-A, which may contribute to their antidepressant effect. Vortioxetine, paroxetine, and sertraline showed significant inhibition of MAO-B, which could be used therapeutically in neurodegenerative disorders by reducing ROS production during oxidation of monoamine neurotransmitters. These results suggest that MAO inhibition may play a role in both the antidepressant and neuroprotective effects of the tested antidepressants.

Our research suggests that pharmacologically distinct antidepressants directly target mitochondria as part of their mechanism of action, suggesting specific selectivity in the interactions between antidepressants and mitochondria. The decrease in respiratory rate induced by antidepressants may be related to the adverse effects associated with antidepressant therapy. However, it is clear that the therapeutic effects of antidepressants are associated with long-term adaptive changes in neurotransmission. Therefore, the initial decrease in respiratory rate induced by antidepressants may serve as a starting point for complex cellular responses within the intracellular environment, that ultimately lead to adaptive changes that support neuroplasticity. We can hypothesize that mild antimitochondrial effects of antidepressants may provide a potentially protective preconditioning effect. However, the effects of antidepressants on cognition are complex, involve multiple mechanisms, and vary by drug and patient population.

4. Conclusion

Our *in vitro* studies with antipsychotics and antidepressants revealed important and statistically significant drug-induced changes in selected mitochondrial parameters, especially at high concentrations (50-100 μM). These findings support the key role of mitochondria in the pathophysiology of psychiatric disorders.

Most of the tested psychopharmaca significantly inhibited the activity of mitochondrial enzymes and mitochondrial respiration, with complex I, IV, and complex I-linked respiration being the most affected parameters. No effect was observed on the activity of citrate synthase or malate dehydrogenase. Although there is a strong correlation between

the activity of ETC complexes and complex I-linked respiration, this correlation is not unequivocal.

Prolonged inhibition of OXPHOS could potentially lead to a neuronal ATP deficit and contribute to neuronal damage at very high drug concentrations. Mitochondrial inhibitory effects may be associated with the adverse effects of antidepressants and antipsychotics. However possible activation of mitochondrial compensatory mechanisms and MAO inhibition may contribute to the therapeutic effects of antidepressants and antipsychotics. The precise molecular mechanisms by which various psychopharmaceutic affect other mitochondrial function, and whether mitochondrial dysfunction is primarily a cause, or a consequence of psychiatric disorders, remain to be determined in further clinical research.

5. References

1. Allen, J., R. Romay-Tallon, K. J. Brymer, H. J. Caruncho and L. E. Kalynchuk (2018). "Mitochondria and Mood: Mitochondrial Dysfunction as a Key Player in the Manifestation of Depression." Front Neurosci 12: 386.
2. Barrie Kitto, G. (1969). [19] Intra- and extramitochondrial malate dehydrogenases from chicken and tuna heart: [EC 1.1.1.37 l-Malate: NAD oxidoreductase]. Methods in Enzymology, Academic Press. 13: 106-116.
3. Ben-Shachar, D. (2020). "The bimodal mechanism of interaction between dopamine and mitochondria as reflected in Parkinson's disease and in schizophrenia." J Neural Transm (Vienna) 127(2): 159-168.
4. Corponi, F., C. Fabbri, I. Bitter, S. Montgomery, E. Vieta, S. Kasper, S. Pallanti and A. Serretti (2019). "Novel antipsychotics specificity profile: A clinically oriented review of lurasidone, brexpiprazole, cariprazine and lumateperone." Eur Neuropsychopharmacol 29(9): 971-985.

5. Del Campo, A., C. Bustos, C. Mascayano, C. Acuña-Castillo, R. Troncoso and L. E. Rojo (2018). "Metabolic Syndrome and Antipsychotics: The Role of Mitochondrial Fission/Fusion Imbalance." Front Endocrinol (Lausanne) 9: 144.
6. Devine, M. J. and J. T. Kittler (2018). "Mitochondria at the neuronal presynapse in health and disease." Nature Reviews Neuroscience 19(2): 63-80.
7. Egashira, T., F. Takayama and Y. Yamanaka (1999). "The inhibition of monoamine oxidase activity by various antidepressants: differences found in various mammalian species." Jpn J Pharmacol 81(1): 115-121.
8. Fasipe, O. J. (2019). "The emergence of new antidepressants for clinical use: Agomelatine paradox versus other novel agents." IBRO Rep 6: 95-110.
9. Filiou, M. D. and C. Sandi (2019). "Anxiety and Brain Mitochondria: A Bidirectional Crosstalk." Trends Neurosci 42(9): 573-588.
10. Fisar, Z. (2010). "Inhibition of monoamine oxidase activity by cannabinoids." Naunyn Schmiedebergs Arch Pharmacol 381(6): 563-572.
11. Fišar, Z. (2023). "Biological hypotheses, risk factors, and biomarkers of schizophrenia." Prog Neuropsychopharmacol Biol Psychiatry 120: 110626.
12. Fišar, Z. and J. Hroudová (2016). "Pig Brain Mitochondria as a Biological Model for Study of Mitochondrial Respiration." Folia Biol (Praha) 62(1): 15-25.
13. Folbergrová, J., P. Jesina, R. Haugvicová, V. Lisý and J. Houstek (2010). "Sustained deficiency of mitochondrial complex I activity during long periods of survival after seizures induced in immature rats by homocysteic acid." Neurochem Int 56(3): 394-403.
14. Glassman, A. H. and J. T. Bigger, Jr. (2001). "Antipsychotic drugs: prolonged QTc interval, torsade de pointes, and sudden death." Am J Psychiatry 158(11): 1774-1782.
15. Grimm, S. (2013). "Respiratory chain complex II as general sensor for apoptosis." Biochim Biophys Acta 1827(5): 565-572.
16. Howes, O., R. McCutcheon and J. Stone (2015). "Glutamate and dopamine in schizophrenia: an update for the 21st century." J Psychopharmacol 29(2): 97-115.

17. Hroudova, J. and Z. Fisar (2010). "Activities of respiratory chain complexes and citrate synthase influenced by pharmacologically different antidepressants and mood stabilizers." Neuro Endocrinol Lett 31(3): 336-342.
18. Hroudová, J. and Z. Fišar (2011). "Connectivity between mitochondrial functions and psychiatric disorders." Psychiatry Clin Neurosci 65(2): 130-141.
19. Hroudová, J. and Z. Fišar (2012). "In vitro inhibition of mitochondrial respiratory rate by antidepressants." Toxicol Lett 213(3): 345-352.
20. Chen, R., H.-A. Park, N. Mnatsakanyan, Y. Niu, P. Licznerski, J. Wu, P. Miranda, M. Graham, J. Tang, A. J. W. Boon, G. Cossu, W. Mandemakers, V. Bonifati, P. J. S. Smith, K. N. Alavian and E. A. Jonas (2019). "Parkinson's disease protein DJ-1 regulates ATP synthase protein components to increase neuronal process outgrowth." Cell Death & Disease 10(6): 469.
21. Jou, S. H., N. Y. Chiu and C. S. Liu (2009). "Mitochondrial dysfunction and psychiatric disorders." Chang Gung Med J 32(4): 370-379.
22. Liu, Y., G. Fiskum and D. Schubert (2002). "Generation of reactive oxygen species by the mitochondrial electron transport chain." J Neurochem 80(5): 780-787.
23. Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall (1951). "Protein measurement with the Folin phenol reagent." J Biol Chem 193(1): 265-275.
24. Maes, M., Z. Fišar, M. Medina, G. Scapagnini, G. Nowak and M. Berk (2012). "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 20(3): 127-150.
25. Manfredi, G., A. Spinazzola, N. Checcarelli and A. Naini (2001). "Assay of mitochondrial ATP synthesis in animal cells." Methods Cell Biol 65: 133-145.
26. Manji, H., T. Kato, N. A. Di Prospero, S. Ness, M. F. Beal, M. Krams and G. Chen (2012). "Impaired mitochondrial function in psychiatric disorders." Nat Rev Neurosci 13(5): 293-307.

27. Montes, J. M., L. Ferrando and J. Saiz-Ruiz (2004). "Remission in major depression with two antidepressant mechanisms: results from a naturalistic study." J Affect Disord 79(1-3): 229-234.
28. Pathak, R. U. and G. P. Davey (2008). "Complex I and energy thresholds in the brain." Biochim Biophys Acta 1777(7-8): 777-782.
29. Rustin, P., D. Chretien, T. Bourgeron, B. Gérard, A. Rötig, J. M. Saudubray and A. Munnich (1994). "Biochemical and molecular investigations in respiratory chain deficiencies." Clin Chim Acta 228(1): 35-51.
30. Sjövall, F., S. Morota, M. J. Hansson, H. Friberg, E. Gnaiger and E. Elmér (2010). "Temporal increase of platelet mitochondrial respiration is negatively associated with clinical outcome in patients with sepsis." Crit Care 14(6): R214.
31. Speijer, D. (2019). "Can All Major ROS Forming Sites of the Respiratory Chain Be Activated By High FADH(2) /NADH Ratios?: Ancient evolutionary constraints determine mitochondrial ROS formation." Bioessays 41(1): e1800180.
32. Srere, P. A. (1969). [1] Citrate synthase: [EC 4.1.3.7. Citrate oxaloacetate-lyase (CoA-acetylating)]. Methods in Enzymology, Academic Press. 13: 3-11.
33. Toker, L. and G. Agam (2015). "Mitochondrial dysfunction in psychiatric morbidity: current evidence and therapeutic prospects." Neuropsychiatr Dis Treat 11: 2441-2447.
34. Tonkonogi, M. and K. Sahlin (1997). "Rate of oxidative phosphorylation in isolated mitochondria from human skeletal muscle: effect of training status." Acta Physiol Scand 161(3): 345-353.
35. Trounce, I. A., Y. L. Kim, A. S. Jun and D. C. Wallace (1996). "Assessment of mitochondrial oxidative phosphorylation in patient muscle biopsies, lymphoblasts, and transmitochondrial cell lines." Methods Enzymol 264: 484-509.
36. van Rensburg, D. J., Z. Lindeque, B. H. Harvey and S. F. Steyn (2022). "Reviewing the mitochondrial dysfunction paradigm in rodent models as platforms for neuropsychiatric disease research." Mitochondrion 64: 82-102.

37. Wood, S. J., M. Yücel, C. Pantelis and M. Berk (2009). "Neurobiology of schizophrenia spectrum disorders: the role of oxidative stress." Ann Acad Med Singap 38(5): 396-396.

List of publications:

1. Publications *in extenso* related to this thesis

- **Ľupták M**, Fišar Z, Hroudová J. Different effects of SSRIs, bupropion, and trazodone on mitochondrial functions and monoamine oxidase isoform activity. Antioxidants. 2023; 12(6):1208. doi: 10.3390/antiox12061208. (IF 2022 = 7.675)
- **Ľupták M**, Fišar Z, Hroudová J. Agomelatine, Ketamine and Vortioxetine Attenuate Energy Cell Metabolism - *In Vitro* Study. Int J Mol Sci. 2022; 23(22):13824. doi: 10.3390/ijms232213824. (IF 2022 = 6.208)
- **Ľupták M**, Michaličková D, Fišar Z, Kitzlerová E, Hroudová J. Novel approaches in schizophrenia-from risk factors and hypotheses to novel drug targets. World J Psychiatry. 2021; 11(7):277-296. doi: 10.5498/wjp.v11.i7.277. (IF 2021 = 3.5)
- **Ľupták M**, Fišar Z, Hroudová J. Effect of Novel Antipsychotics on Energy Metabolism - In Vitro Study in Pig Brain Mitochondria. Mol Neurobiol. 2021; 58(11):5548-5563. doi: 10.1007/s12035-021-02498-4. (IF 2021 = 5.686)
- Cikánková T, Fišar Z, Bakhouche Y, **Ľupták M**, Hroudová J. In vitro effects of antipsychotics on mitochondrial respiration. Naunyn Schmiedebergs Arch Pharmacol. 2019; 392(10):1209-1223. doi: 10.1007/s00210-019-01665-8. (IF 2019 = 3.195)
- **Ľupták M**, Hroudová J. Important role of mitochondria and the effect of mood stabilizers on mitochondrial

function. Physiol Res. 2019; 68(Suppl 1):S3-S15. doi: 10.33549/physiolres.934324. (IF 2019 = 2.139)

2. Publications *in extenso* unrelated to this thesis

- Michaličková D, Kübra Öztürk H, Hroudová J, **Ľupták M**, Kučera T, Hrnčíř T, Kutinová Canová N, Šíma M, Slanař O. Edaravone attenuates disease severity of experimental auto-immune encephalomyelitis and increases gene expression of Nrf2 and HO-1. Physiol Res. 2022; 71(1):147-157. doi: 10.33549/physiolres.934800. (IF 2022 = 2.139)
- Fišar Z, **Ľupták M**, Hroudová J. Little in vitro effect of remdesivir on mitochondrial respiration and monoamine oxidase activity in isolated mitochondria. Toxicol Lett. 2021; 350:143-151. doi: 10.1016/j.toxlet.2021.07.015.
- Ruda-Kucerova J, Babinska Z, **Luptak M**, Getachew B, Tizabi Y. Both ketamine and NBQX attenuate alcohol drinking in male Wistar rats. Neurosci Lett. 2018; 666:175-180. doi: 10.1016/j.neulet.2017.12.055. (IF 2017 = 2.173)