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Mgr. Matej Ľupták

In vitro účinky vybraných psychofarmak na energetický metabolismus

In vitro effects of selected psychopharmaca on energy metabolism

Doctoral Thesis

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

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Abstrakt

Mitochondriální dysfunkce jsou klíčovým faktorem v patofyziologii psychiatrických onemocnění a mohou sloužit jako modulátor a prediktor terapeutické odpovědi. Porozumění mitochondriálním účinkům antipsychotik a antidepresiv je důležité pro propojení jejich terapeutických a nežádoucích účinků s mitochondriálními funkcemi. V této studii jsme používali jako *in vitro* model mitochondrie izolované z prasečích mozků a hodnotili jsme vliv vybraných antidepresiv a antipsychotik na mitochondriální parametry. Byla testovaná vybraná antipsychotika (aripiprazol, brexpiprazol, haloperidol, chlorpromazin, kariprazin, klozapin, levomepromazin, loxapin, lurasidon, olanzapin, quetiapin, risperidon, ziprasidon a zotepin) a antidepresiva (agomelatin, bupropion, escitalopram, fluvoxamin, ketamin, paroxetin, sertralin a vortioxetin). Byly měřeny aktivity komplexů elektronového transportního řetězce I, II+III a IV, citrátsyntázy, malátdehydrogenázy a monoaminooxidázy, stejně jako mitochondriální respirace a tvorba ATP a peroxidu vodíku. Výsledky ukázaly, že většina testovaných antipsychotik a všechna antidepresiva (kromě bupropionu u komplexu II+III) signifikantně inhibovala aktivitu komplexů elektronového transportního řetězce. Plnými inhibitory mitochondriální respirace přes komplex I byla antipsychotika aripiprazol, haloperidol, kariprazin, quetiapin, risperidon a zotepin; z antidepresiv plně inhiboval vortioxetin. Plnými inhibitory respirace přes komplex II byly antipsychotika klozapin, lurasidon, quetiapin a zotepin. Signifikantní změny v produkci ATP byly pozorovány u antipsychotik brexpiprazolu, kariprazinu, loxapinu a lurasidonu, a všech testovaných antidepresiv. Všechna testovaná antipsychotika a antidepresiva agomelatin a vortioxetin signifikantně ovlivnila produkci peroxidu vodíku. Escitalopram, fluvoxamin a sertralin plně inhibovaly aktivitu monoaminooxidázy A; escitalopram, a paroxetin plně inhibovaly aktivitu monoaminooxidázy B. Výsledky tohoto výzkumu naznačují, že dlouhodobá inhibice oxidativní fosforylace může mít vliv na nedostatek ATP v neuronech a přispívat k poškození neuronů při vysokých koncentracích psychofarmak. Mitochondriální účinky testovaných psychofarmak jsou pravděpodobně spojeny s jejich nežádoucími účinky. Aktivace kompenzačních mitochondriálních mechanismů a inhibice monoaminooxidázy ale mohou hrát roli v terapeutických účincích antipsychotik a antidepresiv.

Klíčová slova: Antidepresiva, Antipsychotika, ATP, Elektronový transportní řetězec, Mitochondriální respirace, Monoaminooxidáza, Oxidativní fosforylace, Reaktivní formy kyslíku

Abstract

Mitochondrial dysfunction is a key factor in the pathophysiology of psychiatric disorders and may serve as a modulator and predictor of therapeutic response. Understanding the mitochondrial effects of antipsychotics and antidepressants is crucial to relate their therapeutic and adverse effects to mitochondrial functions. In this study, we used mitochondria isolated from pig brain as an *in vitro* model and evaluated the effects of selected antidepressants and antipsychotics on mitochondrial parameters. Selected antipsychotics (aripiprazole, brexpiprazole, cariprazine, chlorpromazine, clozapine, haloperidol, levomepromazine, loxapine, lurasidone, olanzapine, quetiapine, risperidone, ziprasidone, and zotepine) and antidepressants (agomelatine, bupropion, escitalopram, fluoxetine, paroxetine, sertraline, and vortioxetine) were tested. The activities of electron transport chain complexes I, II+III, and IV, citrate synthase, malate dehydrogenase, and monoamine oxidases, as well as mitochondrial respiration, ATP production, and hydrogen peroxide formation were measured. The results showed that the majority of tested antipsychotics and all tested antidepressants (except bupropion for complex II+III) significantly inhibited the activity of the electron transport chain complexes. Full inhibitors of complex I-linked respiration were antipsychotics aripiprazole, cariprazine, haloperidol, quetiapine, risperidone, and zotepine; among antidepressants, only vortioxetine. Full inhibitors of complex II-linked respiration were antipsychotics clozapine, lurasidone, quetiapine, and risperidone. Significant changes in ATP production were observed with antipsychotics brexpiprazole, cariprazine, loxapine, lurasidone, and all tested antidepressants. All tested antipsychotics and antidepressants agomelatine, and vortioxetine significantly affected hydrogen peroxide formation. Escitalopram, fluvoxamine, and sertraline fully inhibited monoamine oxidase A activity, while escitalopram and paroxetine fully inhibited monoamine oxidase B activity. The results of this research suggest that long-term inhibition of oxidative phosphorylation may affect ATP deficiency in neurons and contribute to neuronal damage at high concentrations of psychotropic drugs. The mitochondrial effects of the tested psychotropic drugs are likely related to their adverse effects, and the activation of compensatory mitochondrial mechanisms and monoamine oxidase inhibition may play a role in the therapeutic effects of antipsychotics and antidepressants.

Key words: Antidepressants, Antipsychotics, ATP, Electron Transport Chain, Mitochondrial Respiration, Monoamine Oxidase, Oxidative Phosphorylation, Reactive Oxygen Species

Contents

List of abbreviations

AD	antidepressant
AP	antipsychotic
ATP	adenosine triphosphate
BDNF	brain-derived neurotrophic factor
CoQ	oxidized form of coenzyme Q10
$CoQH_2$	reduced form of coenzyme Q10
cyt *c*	cytochrome *c*
ETC	electron transport chain
FAD^+	oxidized form of flavin adenine dinucleotide
$FADH_2$	reduced form of flavin adenine dinucleotide
Fe-S	iron-sulfur clusters
FMN	flavin mononucleotide
IMS	intermembrane space
MAO	monoamine oxidase
MDD	major depressive disorder
mtDNA	mitochondrial DNA
NADH	nicotinamide adenine dinucleotide
NMDA	*N*-methyl-D-aspartate
NS	not significant
NTF	neurotrophic factor
OXPHOS	oxidative phosphorylation
ROS	reactive oxygen species
SSRI	selective serotonin reuptake inhibitor

List of publications

Summary IF = 28.403 (related to dissertation thesis)

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1. Publications *in extenso* related to dissertation 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 2021 = 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 2021 = 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 = 3.500)

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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 2021 = 3.195)

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2. Publications *in extenso* not related to dissertation 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 2021 = 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. (IF 2021 = 4.271)

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Abstracts and posters at experts' conferences

Ľupták M, Fišar Z, Hroudová J. Vliv nových antipsychotik na mitochondriální funkce – *in vitro* studie. Mitochondrial effects of novel antipsychotics – *in vitro* study. 63. Česko-Slovenská psychofarmakologická konference, Jeseník 16. 1. 2021. Psychiatrie 2021; 25 (Supl 1): 31-32, poster.

Cikánková T, **Ľupták M**, Fišar Z, Bakhouche Y, Hroudová J. *In vitro* účinek antipsychotik na mitochondriální respiraci. *In vitro* effects of antipsychotics on mitochondrial respiration. 62. Česko-Slovenská psychofarmakologická konference, Jeseník 15. - 19. 1. 2020. Psychiatrie 2020; 24 (Supl 1):54, poster.

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1. Introduction

Mitochondria serve primarily as a source of cellular energy through the Krebs cycle and β-oxidation to produce substrates for oxidative phosphorylation (OXPHOS). Mitochondria are one of the largest organelles in human cells. Their surface is covered by the outer mitochondrial membrane, which is easily permeable to solutes up to 5 kDa. The inner mitochondrial membrane is rather impermeable and divides the inner mitochondrial space into two compartments: the intermembrane space (IMS) and the matrix. The surface of the inner mitochondrial membrane is folded in several times to form cristae, which contain many transport channels and integral proteins that enable the formation of adenosine triphosphate (ATP). Frequent fusion (merging) and fission (dividing) is typical for mitochondria, to prevent accumulation of mutated mitochondrial DNA (mtDNA) (Lodish, Berk et al. 2008, Srivastava, Faust et al. 2018) and to ensure the maintenance of a functional mitochondrial network. In addition to their pivotal role in ATP production, mitochondria are also essential for cellular signaling, generation of reactive oxygen species (ROS), regulation of apoptosis, thermoregulation, and regulation of intracellular calcium levels (de Sousa, Machado-Vieira et al. 2014).

Mitochondria are the major source of ROS produced mainly by complexes I and III of the electron transport chain (ETC) and monoamine oxidase (MAO) (Edmondson 2014). Physiologically, ROS are also secondary signaling messengers that link bioenergetics to other body systems such as inflammatory responses, autophagy, or circadian control (Bolaños, Cadenas et al. 2016). Mitochondria are also key players in maintaining calcium homeostasis, serving as a transient high-capacity calcium store, by forming inorganic phosphates (Feissner, Skalska et al. 2009). Mitochondria also contribute to apoptosis through both intrinsic and extrinsic pathways. Mitochondrial dysfunctions include decreased ATP production, activation of the intrinsic apoptotic pathway, increased oxidative stress, elevated calcium levels, and damaged mtDNA. The extrinsic pathway involves the formation of the death-inducing signaling complex, which leads to the activation of caspases that trigger programmed cell death (Green and Kroemer 1998, Kroemer, Galluzzi et al. 2007, Vakifahmetoglu-Norberg, Ouchida et al. 2017).

If not effectively neutralized by the antioxidant system, ROS overload can cause oxidative damage to cellular lipids, proteins, and nucleic acids. ROS overproduction has been linked to the induction of apoptosis, necrotic cell death and other pathophysiological consequences (Lanciano, Khalfaoui-Hassani et al. 2013).

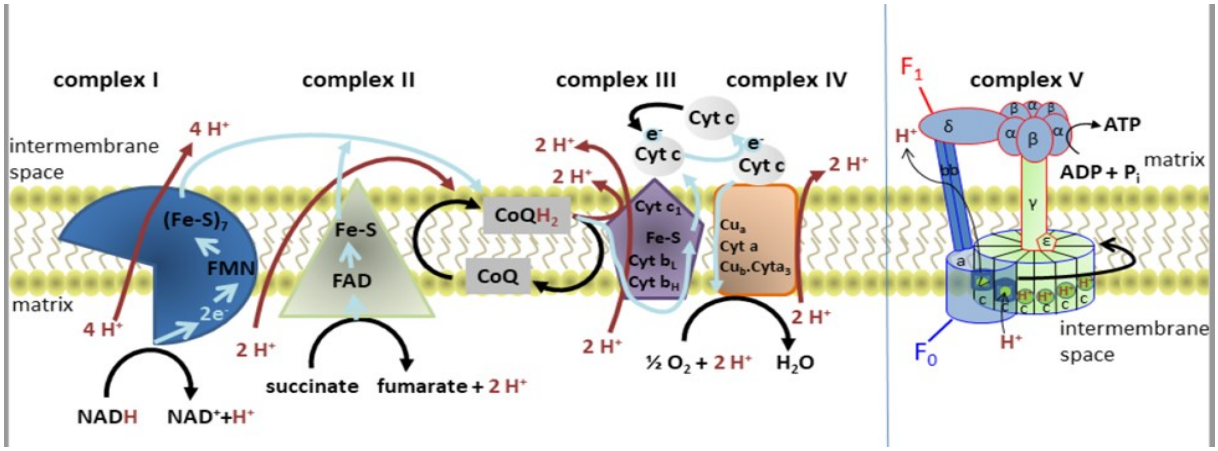
1.1. Role of mitochondria in energy cell metabolism

The primary function of mitochondria in cellular energy metabolism is to utilize products of glycolysis, proteolysis, or lipolysis, along with the oxygen, to generate ATP by OXPHOS using ETC complexes (Clay, Sillivan et al. 2011, Andreazza and Nierenberg 2018). Nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide ($FADH_2$), reduced forms of coenzymes, produced during the breakdown of glucose, fatty acids, and other fuel molecules, donate the electrons to the ETC (de Sousa, Machado-Vieira et al. 2014). Mitochondria operate on electron transfer reactions, where electrons move from acceptors of lower electronegativity to those of higher electronegativity, carried out by redox reactions. The energy released from these redox reactions is used to pump H^+ ions into the IMS, leading to generation of the H^+ concentration gradient (ΔpH) and electric potential gradient (the proton-motive force) that drives ATP synthesis (Lodish, Berk et al. 2008, Divakaruni and Brand 2011).

The ECT consists of four complexes with supramolecular organization, each containing prosthetic groups such as heme or iron-sulfur clusters. Complexes I, III, and IV are responsible for pumping H^+ ions into the IMS (Lodish, Berk et al. 2008, Ohnishi, Ohnishi et al. 2018). Complex I (NADH-coenzyme Q oxidoreductase) is a large multisubunit enzyme and the major entry point into the OXPHOS that catalyzes the regeneration of reduced NADH by transferring electrons from NADH to flavin mononucleotides (FMN) and further through iron-sulfur clusters to oxidized coenzyme Q10 (ubiquinone, CoQ), which is reduced to ubiquinol (Hirst 2009, Ohnishi, Ohnishi et al. 2018). Psychopharmaca can readily inhibit complex I, making it an interesting target for future research (Hroudova and Fisar 2010). Complex II (succinate dehydrogenase) is a side entry of high-energy electrons into OXPHOS metabolic pathway. It allows $FADH_2$ to provide energetic electrons to the ETC and it is directly involved in the Krebs cycle. It transfers electrons through iron-sulfur clusters to CoQ, reducing it to ubiquinol. However, the energy released in this step is not sufficient to allow pumping of H^+ into the IMS, so this reaction does not generate proton motive force (Lodish, Berk et al. 2008, Grimm 2013). CoQ acts as a major transporter of electrons from complexes I and II to complex III (Rodríguez-Hernández, Cordero et al. 2009). Complex III (coenzyme Q10-cytochrome *c* oxidoreductase) is a transmembrane enzyme complex that catalyzes the oxidoreduction of reduced coenzyme Q10 ($CoQH_2$) and cytochrome *c* (cyt *c*) in a multi-step reaction called the Q cycle. Overall, complex III both generates and utilizes $CoQH_2$

as a part of its role in the ETC (Lodish, Berk et al. 2008, Speijer 2019). Complex IV (cytochrome *c* oxidase) is responsible for the final reduction of oxygen to water. Reduced cyt *c* is bound to complex IV and electrons are transported in the reaction sequence, reducing oxygen to water (Lodish, Berk et al. 2008, Hroudová and Fišar 2013). Complex V (ATP synthase) is the final enzyme in the OXPHOS process. As a biological nanomotor, complex V uses the proton gradient generated by the ETC as an energy source for ATP synthesis. This proton gradient is created by the movement of H^+ ions across the inner mitochondrial membrane, resulting in a higher concentration of H^+ in the IMS and a lower concentration in the mitochondrial matrix. The electrochemical energy generated drives the proton motive force, with H^+ ions returning to the matrix through subunit a of F_O. The rotation mechanism of ATP synthase during ATP synthesis is described by the binding-change mechanism. The three β subunits within F_1 bind adenosine diphosphate and phosphatase, and the "c ring" rotation causes conformational changes in the β subunits, which exhibit three conformations: open, loose, and tight. ATP is synthesized during a complete 360° rotation. The synthesis and hydrolysis of ATP depend on the direction of rotation (Lodish, Berk et al. 2008, Neupane, Bhuju et al. 2019). An alternative rotation model inspired by a retractable click ballpoint pen has been proposed, suggesting that the α3β3 hexamer rotates instead of the "c ring" (Liu, Fu et al. 2016). The mitochondrial electron transport chain and the structure of ATP synthase are depicted in Figure 1.

Figure 1. Electron transport chain diagram and structure of ATP synthase



Electron flow in the mitochondrial electron transport chain (ETC) begins with reduced nicotinamide adenine dinucleotide (NADH) donating electrons (indicated by blue arrows) through complex I, involving flavin mononucleotide (FMN) and iron-sulfur clusters (Fe-S). The electrons combine with oxidized coenzyme Q (CoQ) and two protons to form reduced coenzyme Q ($CoQH_2$). This process results in the transport of four H^+ ions (indicated by red arrows) into the intermembrane space (IMS). Complex II serves as an entry point for electrons from succinate (part of Krebs cycle), which pass through oxidized flavin adenine dinucleotide (FAD^+) and Fe-S clusters before forming $CoQH_2$. The electron flow continues from $CoQH_2$ to complex III, followed by cytochrome *c* (cyt *c*) and complex IV. A total of ten H^+ ions are transported into the IMS for each molecule of NADH, while six H^+ ions are transported for each molecule of $FADH_2$. The F_O domain of ATP synthase consists of three a-subunits, three b-subunits, and "c-ring" composed of ten c-subunits. The a-subunit contains H^+ ion half-channel, which facilitates the movement of protons across the membrane. The F_1 domain consists of α- and β-subunit that form hexamer on the γ-subunit that inserts into the "c-ring". Adapted from Ľupták et al (Ľupták and Hroudová 2019).

1.2. Mitochondrial dysfunction and psychiatric disorders

Mitochondria may play a crucial role in the pathogenesis of psychiatric disorders such as depression, anxiety, schizophrenia, and bipolar affective disorder (Hroudová and Fišar 2011). The impaired function of OXPHOS and reduced energy production could easily endanger the brain, which is highly dependent on energy supply. The brain represents about 3 % of body mass but consumes about 20 % of oxygen and 25 % of glucose. Alterations in mitochondrial function could negatively impact brain energy consumption and increase vulnerability to psychiatric disorders (Filiou and Sandi 2019). In summary, 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 ROS. Post-mortem examination of the brains of patients also revealed anatomical and neuroanatomical abnormalities. More data can be found in the relevant literature (Jou, Chiu et al. 2009, Hroudová and Fišar 2011, Manji, Kato et al. 2012, Toker and Agam 2015).

Several studies suggest the possibility that impaired bioenergetics in circulating platelets may project impaired mitochondrial functions in metabolically active organs and help monitor treatment in patients with Alzheimer's disease or depression. In platelets from depressed patients, decreased mitochondrial respiration and a decrease in the maximal capacity of the ETC have been described. Patients with Alzheimer's disease have decreased activity of citric acid cycle enzymes and complexes III and IV, increased levels of nitric oxide (which forms peroxynitrite in reaction with superoxide), and decreased maximal capacity of the ETC. In addition, decreased activity of citrate synthase and increased activity of complex I have been observed in patients with Alzheimer's disease, while increased activity of the complex II has been observed in patients with both Alzheimer's disease and depression (Fišar, Hansíková et al. 2019, Fišar, Jirák et al. 2019, Petrus, Lighezan et al. 2019).

1.3. Biological hypotheses of depressive disorders

Although major depressive disorder (MDD) is a common psychiatric disorder, its underlying pathophysiology remains unclear despite extensive research. The initial understanding of depression is primarily based on the monoamine-deficiency hypothesis, which proposed that depression is associated with deficiencies of three major monoamine neurotransmitters: serotonin, norepinephrine, and dopamine, at neuronal synaptic clefts. However, the monoamine-deficiency hypothesis has been challenged by the fact that initial antidepressant treatments have an efficacy rate of only 60%-65%, with a remission rate of approximately 30% (Montes, Ferrando et al. 2004, Block and Nemeroff 2014). A significant number of patients experience no improvement, even after combining different antidepressant therapies. Another problem is that while antidepressants (ADs) rapidly increase the levels of monoamine neurotransmitter in the central nervous system by blocking transporters, it often takes two weeks or more for their therapeutic effects to appear. Moreover, the ADs used today have different mechanisms of action, and some of them are very weak inhibitors of even enhancers of monoaminergic transmission. This evidence suggests that monoamine deficiency is only a partial explanation for the development of depression. Currently, numerous basic clinical studies have proposed new

hypotheses regarding the pathogenesis of MDD (Hindmarch 2001, Cai, Huang et al. 2015). The original monoamine hypothesis and two other hypotheses (neurotrophic and mitochondrial hypotheses) are presented below. These hypotheses are the cornerstone of our research.

1.3.1. Monoamine hypothesis

The brain consists of a large population of serotonergic, dopaminergic, and noradrenergic neurons, that play a critical role in memory, behavioral regulation, and mindfulness. These processes are regulated by the prefrontal cortex via noradrenergic neurons (Girotti, Adler et al. 2018). The monoaminergic system is closely associated with several behavioral symptoms observed in depression, such as fatigue, low mood, psychomotor retardation, decreased motivation, and decreased vigilance. Studies have reported lower levels of serotonin in the brains of depressed patients, particularly in suicidal cases. Abnormalities in dopamine levels can lead to impaired motivation and concentration, while reduced levels of norepinephrine, alone or along with dopamine, contribute to aggression, sexual dysfunctions, impaired concentration, appetite changes, mindfulness, and motivation (Brigitta 2002). The monoamine hypothesis was formulated in 1950s and 1960s and theorized that decreased availability of these neurotransmitters impairs cognitive function and neurotransmission, ultimately leading to depression (Schildkraut 1965, Coppen 1967). In addition, disruption of monoamine transporters or receptors may also underlie depression, as studies have shown reduced levels of monoamine neurotransmitters due to decreased numbers of transport proteins at cellular junctions in individuals with depression (Hindmarch 2001, Stockmeier and Rajkowska 2004, Liu, Zhao et al. 2018, Zhou, Xiao et al. 2019). This theory was supported by the observation that drugs that deplete extracellular monoamine levels, such as reserpine, produce depressive symptoms. Further support for the monoamine hypothesis of depression came from the serendipitous discovery that iproniazid, an antimicrobial agent, improved mood in tuberculosis patients with depression. Isoniazid and its derivative iproniazid were found to inhibit MAO, a mitochondrial enzyme responsible for breaking down monoamines in the presynaptic nerve terminal. This inhibition prevented the breakdown of serotonin and norepinephrine. Subsequent studies showed that iproniazid was also effective in treating depression in non-tuberculosis patients, leading to the development of other MAO inhibitors for the treatment of depression. Interestingly, these

drugs increased brain levels of norepinephrine and serotonin, which correlated with behavioral activation (Hirschfeld 2000).

According to advanced monoamine theory, serotonin or norepinephrine levels in the brain are regulated by MAO-A activity mainly, and severity of symptoms of depression is related to changes in the activity of monoamine transporters in specific brain regions (Meyer, Ginovart et al. 2006).

1.3.2. Neurotrophic hypothesis

The neurotrophic factors (NTFs), such as brain-derived neurotrophic factor (BDNF), neurotrophins NT3, NT4, and NT5, are a family of growth factors that support neuronal survival, differentiation, growth, synaptic transmission, and plasticity (Holtzman and Mobley 1994, Amidfar, Réus et al. 2021). BDNF, one of the most studied NTFs, is an important cell survival factor involved in several pathological situations. It plays a critical role in synaptic growth, neuronal survival, neuronal proliferation, and differentiation. It also regulates synaptic morphology, information transmission (by facilitating the release of glutamate, GABA, serotonin, and dopamine), and plasticity, thereby ameliorating depressive symptoms (Duman, Heninger et al. 1997, Lu 2003, Almeida, Manadas et al. 2005, Amidfar, Réus et al. 2021).

The neurotrophic hypothesis of depression proposes that NTFs promote synaptic growth and maintain neuronal survival, whereas their deficiency leads to brain structural atrophy and MDD. ADs act by increasing NTF levels, enhancing synaptic plasticity, and promoting neuronal survival. (Lu 2003, Almeida, Manadas et al. 2005).

There is an important link between the NTF system and stress actions. BDNF shields neurons from different types of neurotoxicity (glutamate, oxidative, or post-corticoid elevation-mediated) (Amidfar, Réus et al. 2021). Different types of stress decreased the expression of BDNF and decreased the levels of NTFs receptors (TrkA, TrkB, and TrkC). The mechanism underlying the effects of stress on BDNF expression involves several factors. Corticosterone, a stress hormone, has been shown to decrease BDNF expression, suggesting its involvement in mediating the stress response. However, removal of the adrenal glands, where corticosterone is produced, does not prevent BDNF downregulation, suggesting the involvement of other factors. Activation of serotonin 5-HT_2 receptors leads to a rapid decrease in BDNF expression, which can be blocked by serotonin receptor antagonists. Cytokines, such as IL-1β, may also mediate BDNF downregulation and its blockade prevents social stress-induced BDNF downregulation.

Stress-induced BDNF expression has implications for both, neuronal function, and morphology. Stress affects neurogenesis in the adult hippocampus, leading to a decrease in the proliferation of new neurons. In animal models of depression, stress-induced reductions in neurogenesis correlate with behavioral despair, which is reversed by antidepressant treatment. Glucocorticoids and *N*-methyl-D-aspartate (NDMA) receptors are also play a role in the effects of stress on neurogenesis, with glucocorticoid administration and NMDA receptor activation decreasing neurogenesis and adrenalectomy and NMDA antagonists increasing neurogenesis (Duman 2004).

Research has highlighted the importance of NTFs, particularly BDNF, and impaired synaptic plasticity as common pathways in depression (Neto, Borges et al. 2011). Reduced hippocampal BDNF mRNA levels have been observed in depressed animal models, and untreated depressed patients often have decreased serum BDNF levels (Russo-Neustadt, Ha et al. 2001, Karege, Perret et al. 2002). In addition, patients with depression often show atrophy or reduced neuron counts in brain regions such as the hippocampus and cerebral cortex (Duman, Malberg et al. 2000). Studies have shown that long-term antidepressant treatment increases BDNF levels in the limbic system and in plasma. In addition, direct administration of BDNF to the brain has shown antidepressant-like effects (Nibuya, Morinobu et al. 1995, Siuciak, Lewis et al. 1997). BDNF has been used as a biomarker for depression, and variations in the BDNF gene have been associated with increased suicide risk in depressed patients (Sarchiapone, Carli et al. 2008). Two factors are thought to contribute to the delayed onset of action of ADs. First, receptor sensitivity adaptation, such as desensitization of presynaptic serotonin 5-HT_{1A} autoreceptors, typically takes two to three weeks (Bortolozzi, Castañé et al. 2012). Second, increased synthesis of cAMP response element-binding protein and BDNF also takes two to three weeks (Duman and Li 2012). However, NTFs are considered a valuable clue to understanding the pathogenesis of depression and the mechanism of action of ADs (Altar 1999).

Overall, understanding the mechanisms of the effect of stress on BDNF synthesis, release, and function is critical for understanding the pathology of MDD and for the development of novel ADs. Several factors, including inflammatory cytokines, glutamate receptors, and hypothalamic-pituitary-adrenal axis hyperactivity, can affect BDNF synthesis, release, and function (Duman 2004, Cai, Huang et al. 2015).

1.3.3. Mitochondrial hypothesis

Although the neurotrophic hypothesis of depression is well established, it remains controversial. Depressive symptoms can manifest even in the absence of reduced neuronal proliferation, and the therapeutic effects of the ADs do not always correlate with increased hippocampal neuronal numbers. Instead, emerging evidence suggests that changes in dendritic complexity and neuronal remodeling may be more closely associated with depression and the efficacy of antidepressant treatment (Allen, Romay-Tallon et al. 2018).

A single resting cortical neuron consumes approximately 4.7 billion ATP molecules per second (Zhu, Qiao et al. 2012). Any disruption in mitochondrial function, impaired OXPHOS function, and resulting reduced energy production can negatively impact the brain's energy use and increase its susceptibility to psychiatric disorders (Filiou and Sandi 2019). Studies have reported mitochondrial mutations and alterations in depression, as well as comorbidity between depression and mitochondrial disease (Munakata, Fujii et al. 2007, Ben-Shachar and Karry 2008, Koene, Kozicz et al. 2009). 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 symptoms (Onishi, Kawanishi et al. 1997, Moretti, Gorini et al. 2003, Fattal, Budur et al. 2006, Devine and Kittler 2018).

Several brain regions have shown significant pathological changes in patients with MDD (Rigucci, Serafini et al. 2010). The amygdala, a critical structure involved in emotional processing, has been found to exhibit alterations in regional blood flow and glucose metabolism associated with the severity of depression (Drevets 1998).

Observational studies have linked mtDNA to depression, highlighting the role of genetic factors (Lesch 2004). The prevalence of depression in patients with mitochondrial disease can be as high as 54% (Allen, Romay-Tallon et al. 2018). Depressed patients have higher rates of mtDNA deletions and lower mtDNA copy numbers. Specific mitochondrial genes have been implicated in depression, affecting oxidative stress and neurotransmission (Gardner, Johansson et al. 2003, Chang, Jou et al. 2015, Wang and Dwivedi 2017, Petschner, Gonda et al. 2018). Alterations in translational products related to mitochondrial function, and changes in mitochondria-associated proteins have been identified in the cortex of postmortem brains from individuals with MDD (Karry, Klein et al. 2004, Beasley, Pennington et al. 2006).

Several animal models of depression have shown alterations in specific proteins related to the OXPHOS, which are further influenced by antidepressant treatment (Zubenko, Hughes et al. 2014, Carboni 2015). Proteomic analysis of the cortex of depressed patients revealed altered proteins associated with metabolic and energy pathways (Johnston-Wilson, Sims et al. 2000, Halari, Simic et al. 2009, Martins-de-Souza, Guest et al. 2012). PET studies have shown reduced cerebral glucose metabolism in depressed patients, which can be reversed by ADs (Baxter, Schwartz et al. 1989, Kennedy, Evans et al. 2001).

Research has consistently shown that depression is associated with decreased ATP levels. Preclinical animal models of depression have shown decreased ATP production (Gamaro, Streck et al. 2003). In individuals with depression, brain ATP levels are generally lower compared to controls (Moretti, Gorini et al. 2003, Martins-de-Souza, Guest et al. 2012, Caruncho, Brymer et al. 2016). Gardner et al. reported significantly decreased mitochondrial ATP production rates and altered enzyme ratios in the ETC complexes I-IV in the muscles of depressed patients. Correlations between these biochemical changes and depression rating scales provide additional evidence linking mitochondrial dysfunction to psychopathology (Gardner, Johansson et al. 2003). In peripheral blood mononuclear cells, depressed patients showed reduced ATP turnover-related respiration, as well as reduced routine and uncoupled respiration and coupling efficiency compared to age-matched controls (Karabatsiakis, Böck et al. 2014).

Mitochondria are the main source of ROS, but they also have protective mechanisms to dispose of excess and harmful free radicals (Petschner, Gonda et al. 2018). Oxidative stress occurs due to the premature electron leakage occurs in the ETC, resulting in mitochondrial and mtDNA damage. Reduced ATP levels in depression may be a consequence of oxidative stress, with increased levels of OXPHOS subunits serving as a compensatory mechanism (Martins-de-Souza, Guest et al. 2012, Vakifahmetoglu-Norberg, Ouchida et al. 2017). Decreased activity of complexes I-III was found after seven days of restraint stress in rats (Madrigal, Olivenza et al. 2001). Similarly, 40 days of chronic variable stress caused inhibition of complex I, II, and IV activity in rats (Rezin, Cardoso et al. 2008). Increased oxidative damage and changes in complex I have been observed in the prefrontal cortex of depressed patients (Ben-Shachar and Karry 2008). Other research has linked depression to reduced antioxidant levels and deficits in the antioxidant systems (Anderson 2018).

1.4. Biological hypotheses of schizophrenia

Similar to depression, the pathophysiology of schizophrenia is not fully understood. It is thought to be caused by abnormalities in neural circuit development and signaling in the brain. These abnormalities result from abnormal activity in the neurotransmitter receptor system and its downstream signaling pathways (Fišar 2023). Various hypotheses have been proposed to explain the neuropathology of schizophrenia, considering environmental, genetic, neurodevelopmental, and neurochemical factors (Ľupták, Michaličková et al. 2021).

According to the classical dopamine hypotheses, psychotic symptoms are associated with excessive dopaminergic activity and increased sensitivity of dopamine D_2 receptors (Meltzer and Stahl 1976). The modified dopamine hypothesis suggests that schizophrenia is characterized by low prefrontal (negative symptoms) and excessive mesolimbic (positive symptoms) activity of dopamine neurons (Davis, Kahn et al. 1991). The final common pathway hypothesis proposes that multiple environmental, genetic, and other risk factors interact to lead to striatal dopamine dysregulation and leading to psychosis (Howes and Kapur 2009). Existing antipsychotics (APs) primarily target the dopamine D_2 receptor to modulate its function. Studies have shown a non-linear relationship between dopamine D_2 receptor occupancy, clinical response, and side effects of current APs (Nordström, Farde et al. 1993).

The glutamate hypothesis focuses on NMDA receptor dysfunction. Both NMDA receptor dysfunction and impaired presynaptic dopamine synthesis are thought to contribute to the clinical symptoms of schizophrenia (Howes, McCutcheon et al. 2015). Developmental abnormalities in glutamate synapse formation, particularly at GABA interneurons in the cerebral cortex, result in excessive glutamate signaling to the ventral tegmental area, which in turn may lead to an overabundance of dopamine in the ventral striatum via the mesolimbic pathway (Stahl 2018). Associations are expected between presynaptic dopamine dysfunction and positive symptoms, and between glutamate dysfunction and negative and cognitive symptoms (Howes, McCutcheon et al. 2015).

1.4.1. Mitochondrial hypothesis

The mitochondrial hypothesis of schizophrenia proposes that mitochondrial dysfunction leads to impaired neuronal activity and plasticity, resulting in disrupted brain circuitry and subsequent abnormal behavior (Ben-Shachar 2020). Mitochondrial

dysfunction and oxidative stress have been implicated in the pathophysiology of schizophrenia (Wood, Yücel et al. 2009, Morris, Walder et al. 2020, van Rensburg, Lindeque et al. 2022). *In vivo* and post-mortem brain imaging studies have demonstrated impaired energy metabolism in individuals with schizophrenia (Fišar 2023).

A prominent feature of schizophrenia is the significant loss of white matter oligodendrocytes. It has been proposed that there is an increase in mitophagy, specifically in oligodendrocytes affected by schizophrenia. This increased mitophagy is thought to contribute to the white matter neuropathology associated with the disease (Ben-Shachar 2020). The oligodendrocyte hypothesis is linked to the glutamate and mitochondrial hypotheses through glutamate metabolism in the brain. Glutamate interacts with ammonia in astrocytes and oligodendrocytes to produce glutamine (since neurons lack the enzymes necessary for this process). Glutamine is then transported into neurons and converted to glutamate in mitochondria. This glutamine cycle serves to prevent the depletion of α-ketoglutarate from the Krebs cycle in neurons when there is excessive release of glutamate from nerve terminals. Disruption of the glutamine cycle leads to mitochondrial dysfunction, oxidative stress, and vice versa (Fišar 2023).

In vitro data suggest that mitochondrial effects of current APs are associated with adverse effects resulting from drug-induced decreased ATP production and increased ROS generation (Cikánková, Fišar et al. 2019, Ľupták, Fišar et al. 2021). Post-mortem studies suggest reduced neuronal and glial density in certain brain regions, supporting the apoptotic hypothesis of schizophrenia (Jarskog, Glantz et al. 2005). Apoptotic activity during the early stages of the disease is thought to contribute to reduced neuronal survival and disruption of synaptic plasticity (Glantz, Gilmore et al. 2006). Cellular stress and mitochondrial dysfunction can initiate the intrinsic apoptotic pathway through the release of proapoptotic factors from mitochondria (Jarskog 2006).

1.5. Effect of psychopharmaca on mitochondrial functions

1.5.1. Antidepressants

In vitro studies have demonstrated the inhibitory effects of certain ADs on mitochondrial complex I. Imipramine, desipramine, amitriptyline, citalopram, and mirtazapine have been found to act as complex I inhibitors. Nefazodone specifically inhibits both complex I and IV, while trazodone did not affect complex I and IV, but reduced oxygen consumption and Na^+/K^+-ATPase activity (Velasco, González-Calvo et

al. 1985, Dykens, Jamieson et al. 2008, Hroudova and Fisar 2010, Cikánková, Fišar et al. 2020). Furthermore, the ADs paroxetine, fluoxetine, and clomipramine have been associated with increased apoptotic markers (Levkovitz, Gil-Ad et al. 2005). Bupropion has been implicated in increasing mitochondrial oxidative stress and inducing apoptotic cell death (Jang, Park et al. 2011, Ahmadian, Babaei et al. 2017). In addition, fluoxetine has shown significant inhibitory effects on the activity of complexes I, II+III, and IV, leading to impaired mitochondrial function in isolated mitochondria (Abdel-Razaq, Kendall et al. 2011). In contrast, amitriptyline, tranylcypromine, and nortriptyline have been observed to exert protective effects on mitochondria by preventing apoptosis (Wang, Guan et al. 2007, Zhang, Wang et al. 2008, Han and Lee 2009). In particular, our research group has previously reported the inhibitory effects of amitriptyline, bupropion, fluoxetine, imipramine, and tianeptine on mitochondrial respiratory rate *in vitro* (Hroudová and Fišar 2012, Cikánková, Fišar et al. 2020).

1.5.2. Antipsychotics

The APs haloperidol, chlorpromazine, fluphenazine, risperidone, and clozapine have been identified as inhibitors of complex I in the human brain (Maurer and Möller 1997, Prince, Yassin et al. 1997). Notably, the activities of complexes II+III and IV were not significantly affected by typical or atypical APs in human brain samples (Hroudova and Fisar 2010). Inhibition of complex I activity has been implicated in the manifestation of extrapyramidal side effects associated with antipsychotic medications (Burkhardt, Kelly et al. 1993, Balijepalli, Kenchappa et al. 2001). In addition, previous studies have demonstrated the inhibitory effects of chlorpromazine on mitochondrial respiratory rate *in vitro* (Hroudová and Fišar 2012).

Detailed information on the effects of the tested antipsychotics on mitochondrial functions is described *in extenso* in the attached publications.

2. Hypotheses and objectives of the work

Mitochondrial dysfunction has been postulated to be a key contributor to the underlying mechanisms of depression and other neuropsychiatric disorders. Disruptions in OXPHOS and ATP production, along with excessive ROS generation and alterations in calcium levels, have the potential to affect neuronal bioenergetics and subsequently impair neuroplasticity and neuronal adaptation. We hypothesize that currently used psychopharmaceuticals for the treatment of these conditions exert effects on mitochondrial function, specifically affecting mitochondrial respiratory efficiency, ATP production, ROS generation, maintenance of calcium homeostasis, as well as apoptosis and neurodevelopment. These drug-induced alterations in mitochondrial function may contribute to both desirable and undesirable treatment outcomes.

Accordingly, the primary objective of this research was to examine the *in vitro* effects of selected psychotropic drugs on cellular energy metabolism. All measurements were performed on an established *in vitro* model of purified mitochondria isolated from pig cerebral cortex. 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 treatment effects.

In order to comprehensively assess the effects of psychopharmaca, we decided to evaluate drugs with different mechanisms of action. As a result, the following drugs were selected for testing:

- Antidepressants: agomelatine, bupropion, escitalopram, fluvoxamine, ketamine, paroxetine, sertraline, and vortioxetine
- Antipsychotics: aripiprazole, brexpiprazole, cariprazine, chlorpromazine, clozapine, haloperidol, levomepromazine, loxapine, lurasidone, olanzapine, quetiapine, risperidone, ziprasidone, and zotepine

The following mitochondrial parameters were evaluated for drug-induced changes:

- Enzyme activities in the citric acid cycle: citrate synthase, malate dehydrogenase
- Enzyme activity of ETC complexes (I, II+III, and IV)

- Complex I-linked and complex II-linked mitochondrial respiration
- ATP formation
- ROS formation
- MAO-A and MAO-B activities

Hypothesis and the aim of the study:

Psychotropic drugs with different mechanisms of action affect mitochondrial functions and modulate neuronal activity.

To verify the proposed hypothesis, the *in vitro* effects of tested psychopharmaca were evaluated in freshly isolated and/or cryopreserved pig brain mitochondria. Mitochondrial respiration was assessed by high-resolution respirometry, while UV/VIS spectrophotometry was used to determine the activity of ETC complexes I, II+II, and IV, as well as the activities of citrate synthase and malate dehydrogenase. Luminescence-based techniques were used to detect ATP levels and kinetics and to assess ROS formation. In addition, the activity of MAO-A and MAO-B activity was assessed using radiolabeled substrates.

3. Methods

All research methods are described *in extenso* in the attached publications.

Pig brains were obtained from a slaughterhouse. 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). 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 (Fišar and Hroudová 2016).

The activities of ETC complexes (I, II+III, and IV), citrate synthase, and malate dehydrogenase were measured spectrophotometrically. The ATP Bioluminescence Assay Kit CLS II was used to quantify the ATP content and assess ATP kinetics. The Amplex Red Hydrogen Peroxide/Peroxidase Assay Kit was used to determine the formation of hydrogen peroxide. The mitochondrial oxygen consumption rate was determined by high-resolution respirometry. The enzymatic activity of MAO was assessed using radiolabeled substrates and the radioactivity of the samples was quantified by liquid scintillation counting.

Mitochondrial enzymes activities and ATP kinetics were assessed by calculating the slope of the absorbance of fluorescence over time. ATP and ROS levels were measured and the average fluorescence curves over time were determined. High-resolution respirometry data showing real-time oxygen concentration and oxygen flux were collected and analyzed using DatLab 7.4 software. Oxygen flux (respiratory rate) was expressed as pmol O_2 consumed per second per mg of a protein. Respiratory rate and MAO activity inhibition were analyzed by a four-parameter logistic regression using Prism software to determine half-maximal inhibitory concentration (IC_{50}), residual activity, and the Hill slope. The IC_{50} represents the concentration of a drug required to inhibit 50 % of the difference between the baseline and the residual value of the mitochondrial oxygen flux or MAO activity.

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.

4. Results

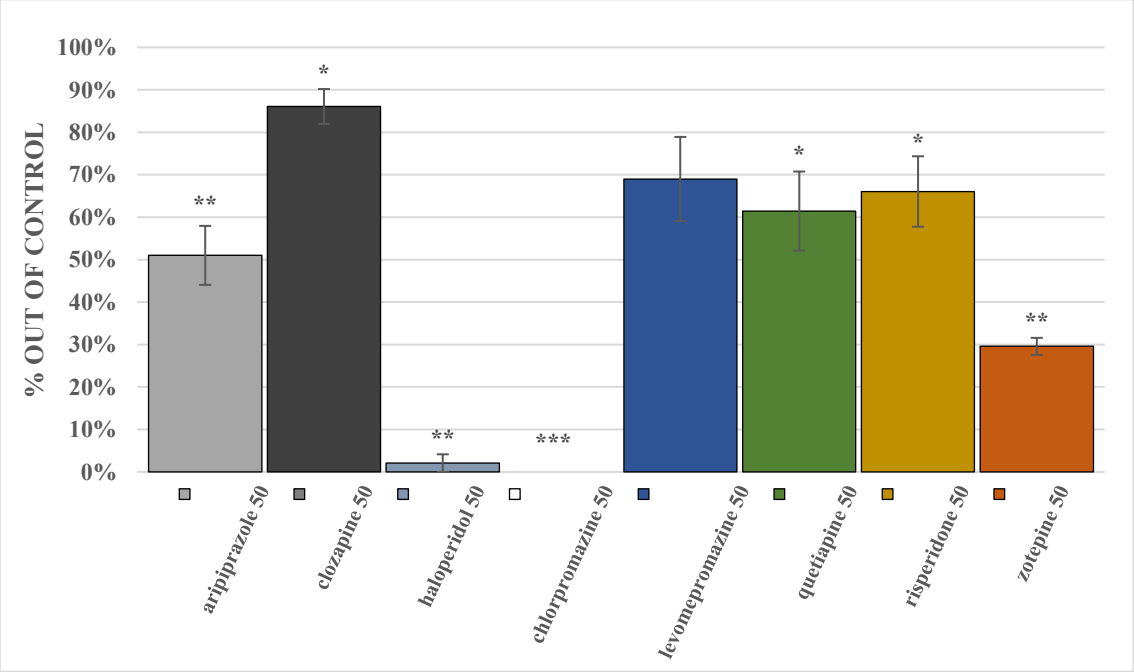
All results are described *in extenso* in the attached publications. Key findings are summarized below.

4.1. Activity of mitochondrial enzymes

4.1.1. Complex I activity

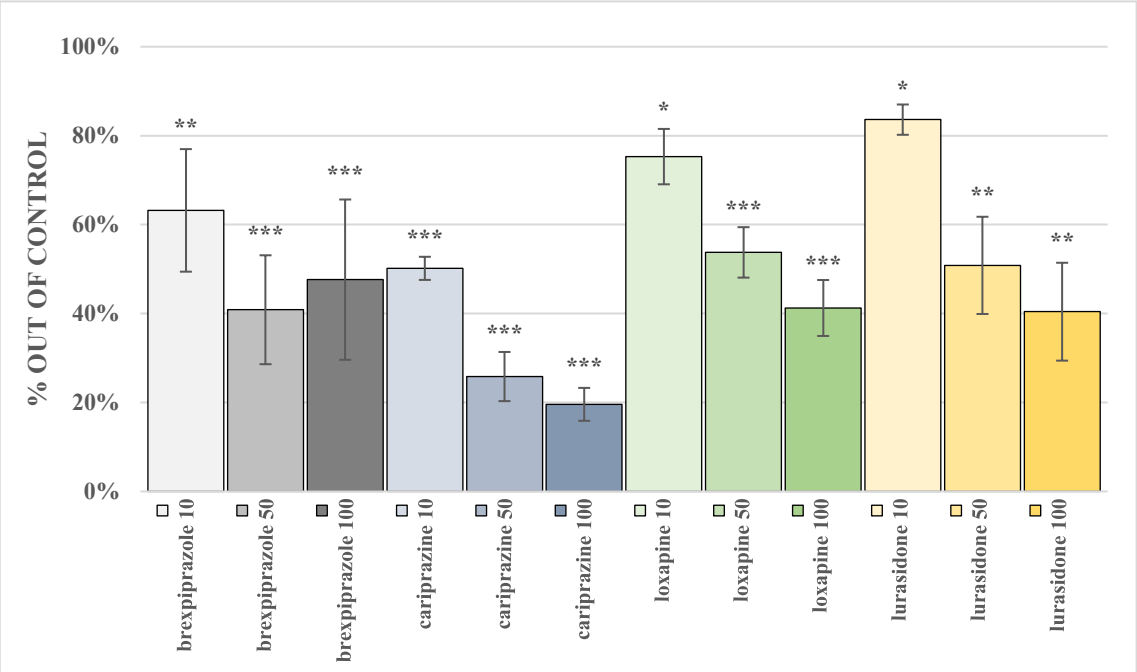
Several tested APs significantly decreased the activity of complex I. The most potent inhibitor was chlorpromazine ($p < 0.001$), which completely stopped the reaction. The inhibitory potential of APs is as follows, from the most potent to the least potent (at 50 µM concentration): chlorpromazine > haloperidol > cariprazine > zotepine > brexpiprazole > lurasidone > aripiprazole > loxapine > quetiapine > risperidone > levomepromazine (not significant) > clozapine > olanzapine (NS) > ziprasidone (NS). The inhibitory activity of selected APs against complex I activity is shown in Figure 2A (at 50 µM concentration) and Figure 2B (at 10-100 µM concentration) (Cikánková, Fišar et al. 2019, Ľupták, Fišar et al. 2021).

Figure 2A. Antipsychotic-induced inhibition of complex I activity



The inhibitory effect of selected antipsychotics on complex I activity was evaluated spectrophotometrically in a purified fraction of pig brain mitochondria. Relative activity is expressed as a percentage difference from the activity of the control sample. A one-sample *t*-test was performed to assess statistical significance, with the control mean set at 100% and is expressed as * $p < 0.05$. ** $p < 0.01$. *** $p < 0.001$. Drug concentrations are expressed as µM.

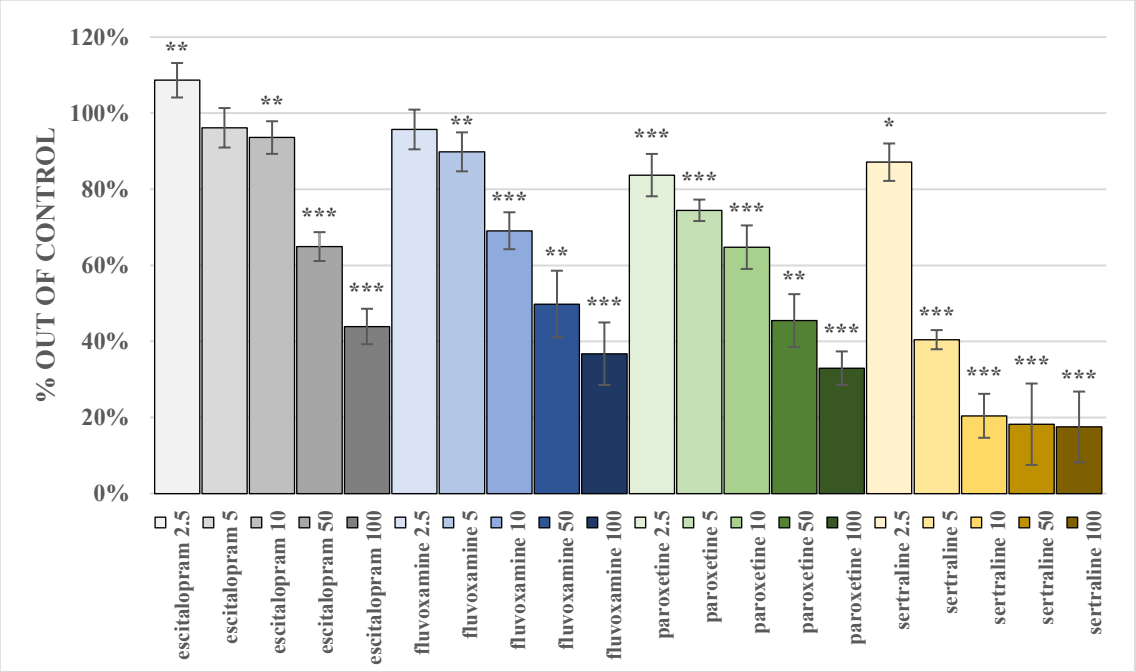
Figure 2B. Inhibition of complex I activity by brexpiprazole, cariprazine, loxapine and lurasidone



The inhibitory effect of selected antipsychotics on complex I activity was evaluated spectrophotometrically in a purified fraction of pig brain mitochondria. Relative activity is expressed as a percentage difference from the activity of the control sample. A one-sample *t*-test was performed to assess statistical significance, with the control mean set at 100% and is expressed as * $p < 0.05$. ** $p < 0.01$. *** $p < 0.001$. Drug concentrations are expressed as µM.

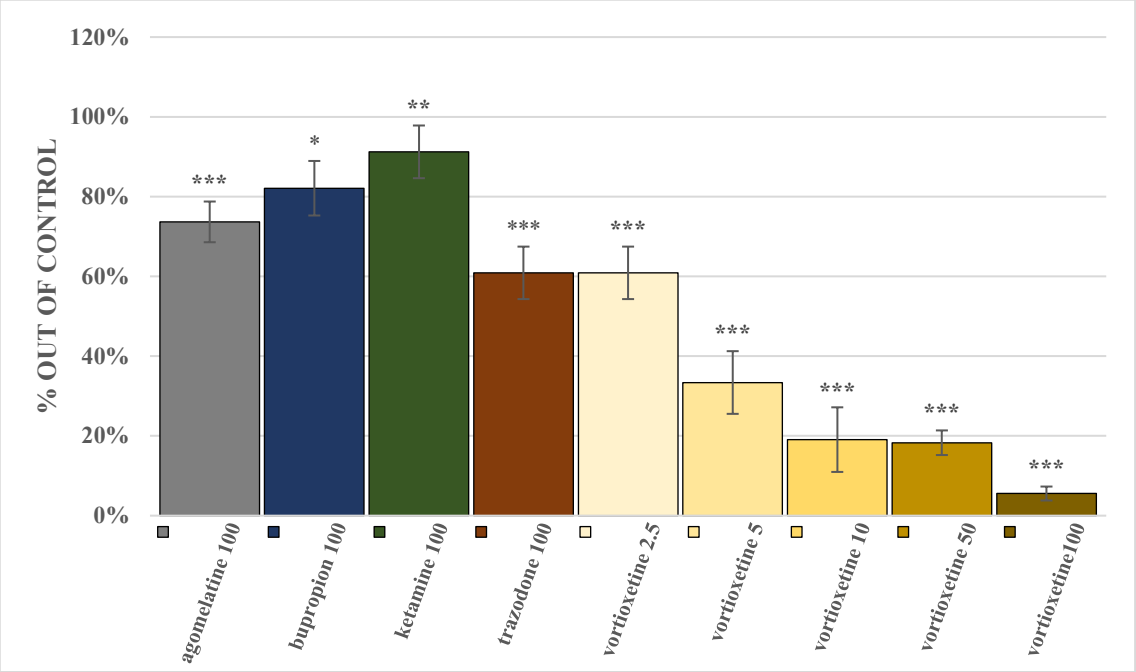
All tested ADs showed significant inhibitory effects against complex I. The most potent inhibitor was vortioxetine (5.5 ± 1.8% at 100 µM, $p < 0.001$). The ADs are listed from the most potent to the least potent (at 100 µM concentration): vortioxetine > sertraline > paroxetine > fluvoxamine > escitalopram > trazodone > agomelatine > bupropion > ketamine. The inhibition of complex I activity by tested ADs (at 2.5-100 µM concentrations) is shown in Figure 2C and 2D (Ľupták, Fišar et al. 2022, Ľupták, Fišar et al. 2023).

Figure 2C. SSRI-induced changes in complex I activity



The inhibitory effect of selected SSRIs on complex I activity was evaluated spectrophotometrically in a purified fraction of pig brain mitochondria. Relative activity is expressed as a percentage difference from the activity of the control sample. A one-sample *t*-test was performed to assess statistical significance, with the control mean at 100% and is expressed as * $p < 0.05$. ** $p < 0.01$. *** $p < 0.001$. Drug concentrations are expressed as µM.

Figure 2D. Inhibition of complex I activity induced by other tested antidepressants



The inhibitory effect of selected antidepressants on complex I activity was evaluated spectrophotometrically in a purified fraction of pig brain mitochondria. Relative activity is expressed as a percentage difference from the activity of the control sample. A one-sample *t*-test was performed to assess statistical significance, with the control mean set at 100% and is expressed as * $p < 0.05$. ** $p < 0.01$. *** $p < 0.001$. Drug concentrations are expressed as µM.

4.1.2. Complex II+III activity

Significant inhibition of complex II+III activity was measured for APs: loxapine, zotepine, lurasidone, aripiprazole, brexpiprazole, cariprazine, quetiapine, and risperidone. The most potent inhibitor of complex II+III activity was loxapine (15.8 ± 8.1% at 100 µM, $p < 0.001$) (Cikánková, Fišar et al. 2019, Ľupták, Fišar et al. 2021).

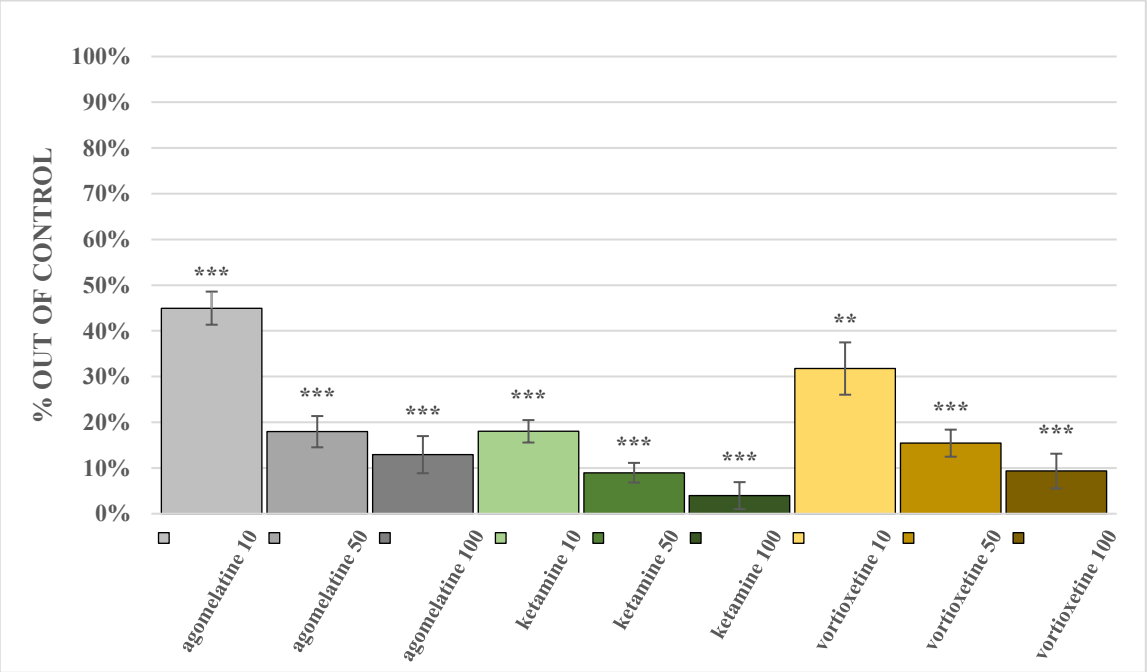
All tested ADs, except bupropion, significantly inhibited complex II+III activity. The most potent inhibitor of complex II+III activity was vortioxetine (43.9 ± 1.2% at 100 µM, $p < 0.001$). The following ADs are listed in descending order of inhibitory potency: vortioxetine > sertraline > paroxetine > trazodone > ketamine ~ agomelatine ~ escitalopram ~ fluvoxamine. Bupropion was the only antidepressant that weakly stimulated complex II+III activity (Ľupták, Fišar et al. 2022, Ľupták, Fišar et al. 2023).

4.1.3. Complex IV

Significant inhibitory activity against complex IV was observed with the following APs: zotepine, chlorpromazine, loxapine (10-50 μM), lurasidone, and levomepromazine. Brexpiprazole (50-100 μM), loxapine (100 μM), and quetiapine significantly increased complex IV activity (Cikánková, Fišar et al. 2019, Ľupták, Fišar et al. 2021).

All tested ADs significantly inhibited complex IV activity, with the greatest potency found for ketamine (3.9 ± 3.0% at 100 μM, $p < 0.001$). Inhibitory potency against complex IV decreased in the following order: ketamine > vortioxetine > agomelatine > trazodone > escitalopram > sertraline > paroxetine > bupropion > fluvoxamine. The inhibitory effects of agomelatine, ketamine, and vortioxetine (at 10-100 μM concentrations) are shown in Figure 3 (Ľupták, Fišar et al. 2022, Ľupták, Fišar et al. 2023).

Figure 3. Inhibition of complex IV activity induced by agomelatine, ketamine, and vortioxetine



The inhibitory effect of selected antidepressants on complex IV activity was evaluated spectrophotometrically in a purified fraction of pig brain mitochondria. Relative activity is expressed as a percentage difference from the activity of the control sample. A one-sample *t*-test was performed to assess statistical significance, with the control mean set at 100% and is expressed as ** $p < 0.01$. *** $p < 0.001$. Drug concentrations are expressed as μM.

4.2. Mitochondrial respiration

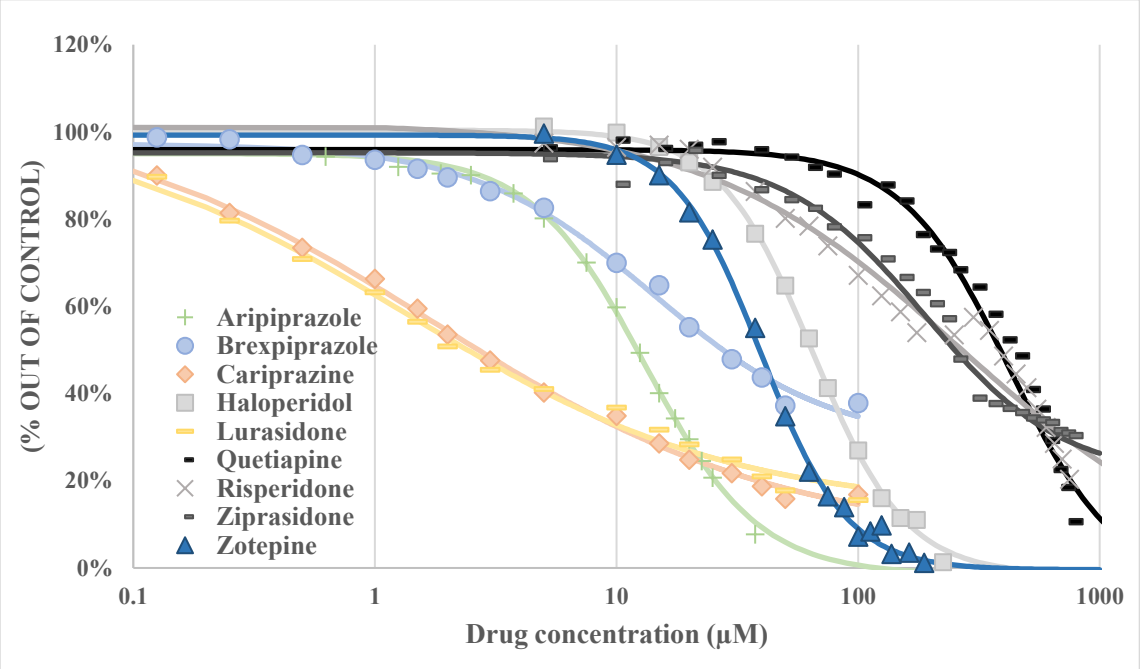
4.2.1. Complex I-linked respiration

Several tested APs significantly inhibited complex I-linked respiration. Cariprazine, aripiprazole, zotepine, haloperidol, quetiapine, and risperidone were identified as full inhibitors of complex I-linked respiration. The most potent inhibitors of complex I-linked respiration were lurasidone (IC_{50} = 1.38 ± 0.25 µM) and cariprazine (IC_{50} = 1.76 ± 0.27 µM) (Cikánková, Fišar et al. 2019, Ľupták, Fišar et al. 2021).

Vortioxetine was the only full inhibitor of complex I-linked respiration and the most potent inhibitor of complex I-linked respiration (IC_{50} = 7.33 ± 1.10 µM) (Ľupták, Fišar et al. 2022, Ľupták, Fišar et al. 2023).

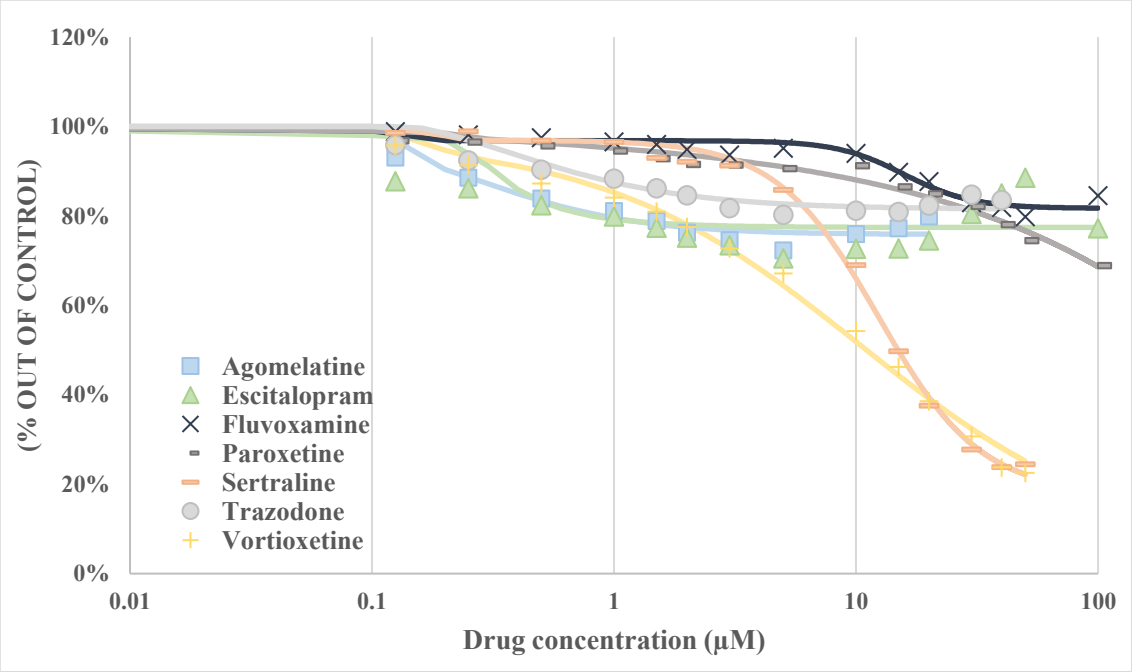
Inhibition curves for selected APs and ADs are shown in Figure 4A and 4B, and the full list of evaluated parameters can be found in the accompanying publications.

Figure 4A. Antipsychotic-induced inhibition of complex I-linked respiration



The inhibitory effect of selected antipsychotics on complex I-linked respiration in a purified fraction of a pig brain mitochondria is shown as dose-response curves, where respiratory rate is plotted against drug concentration on a logarithmic X-axis. Relative activity is expressed as a percentage difference from the activity of the control sample. The lines represent the best-fit curves using a four-parametric logistic function.

Figure 4B. Antidepressant-induced inhibition of complex I-linked respiration



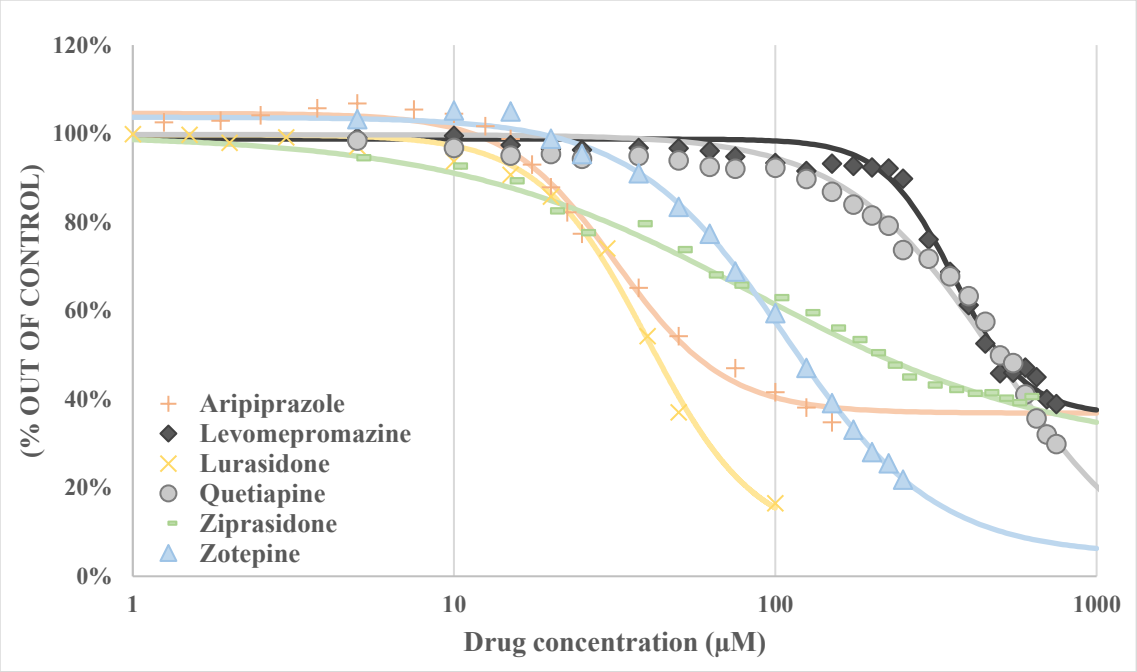
The inhibitory effect of selected antidepressants on complex I-linked respiration in a purified fraction of a pig brain mitochondria is shown as dose-response curves, where respiratory rate is plotted against drug concentration on a logarithmic X-axis. Relative activity is expressed as a percentage difference from the activity of the control sample. The lines represent the best-fit curves using a four-parametric logistic function.

4.2.2. Complex II-linked respiration

The full inhibitors of complex II-linked respiration were antipsychotics lurasidone, zotepine, quetiapine, and clozapine. The most potent inhibitor of complex II-linked respiration was lurasidone (IC_{50} = 39.94 ± 3.19 µM). Inhibition curves for selected APs are shown in Figure 5, and the full list of evaluated parameters can be found in the accompanying publications (Cikánková, Fišar et al. 2019, Ľupták, Fišar et al. 2021).

Bupropion was the only tested antidepressant with inhibitory properties against complex II-linked respiration (IC_{50} = 10.58 ± 4.49 µM), acting as a partial inhibitor (Ľupták, Fišar et al. 2022, Ľupták, Fišar et al. 2023).

Figure 5. Antipsychotic-induced inhibition of complex II-linked respiration

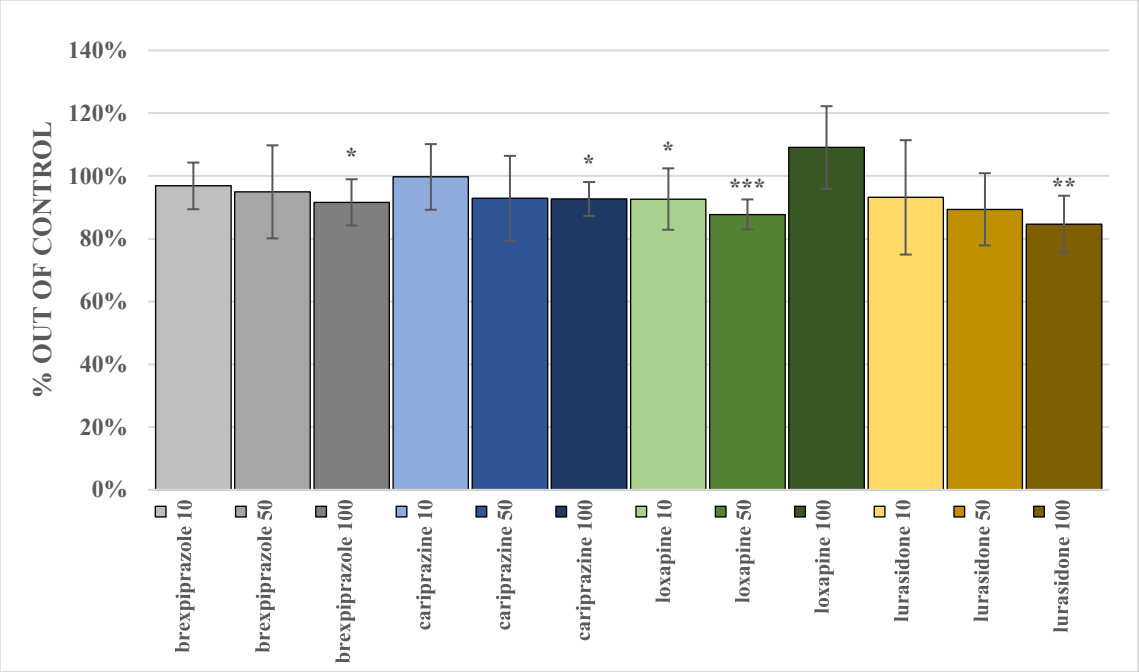


The inhibitory effect of selected antipsychotics on complex I-linked respiration in a purified fraction of a pig brain mitochondria is shown as dose-response curves, where respiratory rate is plotted against drug concentration on a logarithmic X-axis. Relative activity is expressed as a percentage difference from the activity of the control sample. The lines represent the best-fit curves using a four-parametric logistic function.

4.3. ATP production

Significant decreases in ATP formation were observed with brexpiprazole, cariprazine, loxapine, and lurasidone (Figure 6, at 10-100 µM concentrations), with lurasidone causing the greatest decrease in ATP formation (78.7 ± 9.6% at 100 µM, p = 0.009) (Ľupták, Fišar et al. 2021). Vortioxetine (100 µM) significantly increased complex I-linked ATP content and complex II-linked ATP kinetics, and ketamine (10-50 µM) significantly decreased complex II-linked ATP kinetics (Ľupták, Fišar et al. 2022).

Figure 6. Effect of antipsychotics on ATP formation



The effect of selected antipsychotics on ATP formation was evaluated by bioluminescence in a purified fraction of pig brain mitochondria. Relative activity is expressed as a percentage difference from the activity of the control sample. A one-sample *t*-test was performed to assess statistical significance, with the control mean set at 100% and is expressed as * $p < 0.05$. ** $p < 0.01$. *** $p < 0.001$. Drug concentrations are expressed as µM.

Fluvoxamine (50 µM) and trazodone (50 µM) significantly decreased complex I-linked ATP content, and paroxetine (100 µM) significantly decreased complex I-linked ATP kinetics. Complex I-linked ATP content was significantly increased by sertraline (50 µM) and trazodone (10 µM). Complex II-linked ATP content and kinetics were significantly decreased by escitalopram (50 µM), fluvoxamine (50 µM), and paroxetine. Sertraline and trazodone (both at 10 µM) also decreased complex II-linked ATP kinetics. Bupropion (50 µM) was the only antidepressant that slightly stimulated complex II-linked ATP kinetics (Ľupták, Fišar et al. 2023).

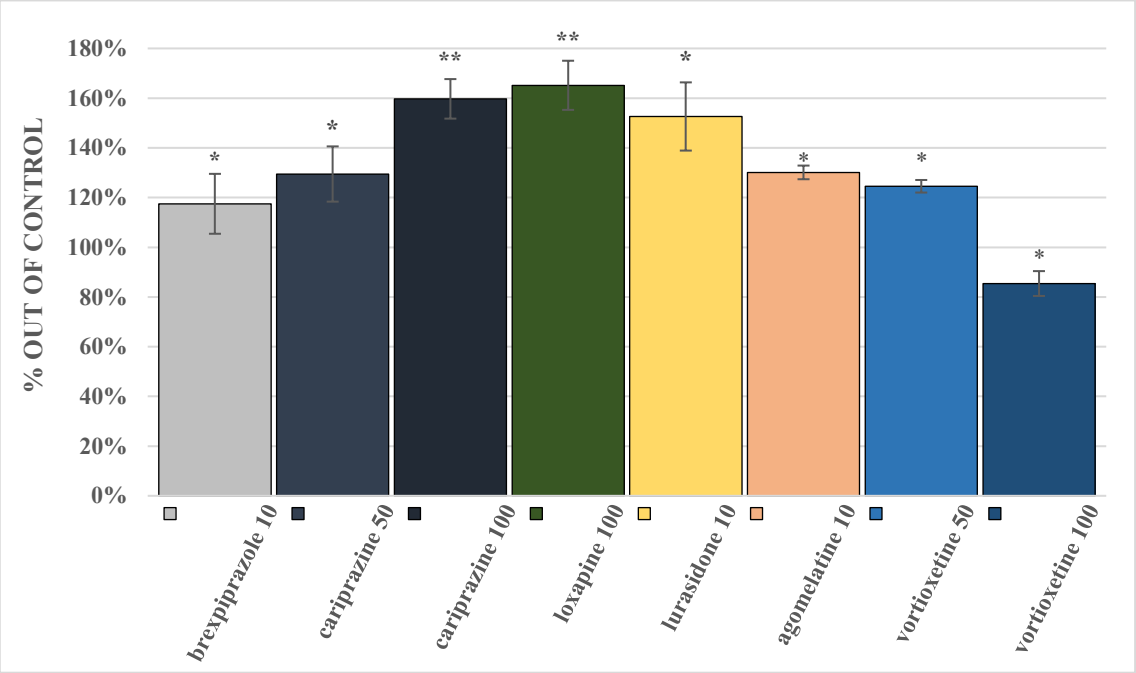
4.4. Hydrogen peroxide formation

All tested APs significantly increased hydrogen peroxide production at various concentration, with loxapine as the most potent stimulator (165.2 ± 9.9% at 100 µM, $p = 0.008$).

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. Psychopharmaca-induced changes in hydrogen peroxide production are shown in Figure 7 (at 10-100 μM concentrations) (Ľupták, Fišar et al. 2021).

Figure 7. Effect of psychopharmaca on hydrogen peroxide production



The effect of selected antipsychotics on hydrogen peroxide formation was evaluated by bioluminescence in a purified fraction of pig brain mitochondria. Relative activity is expressed as a percentage difference from the activity of the control sample. A one-sample t-test was performed to assess statistical significance, with the control mean set at 100% and is expressed as * $p < 0.05$. ** $p < 0.01$. Drug concentrations are expressed as μM.

4.5. MAO activity

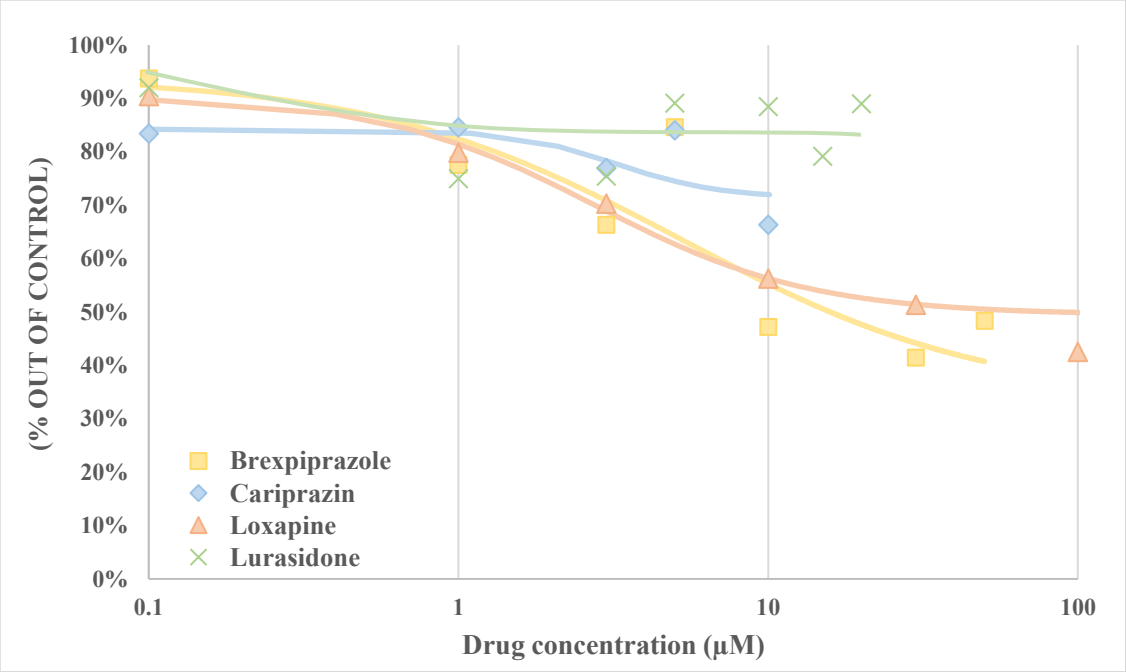
4.5.1. MAO-A activity

Antipsychotics brexpiprazole, cariprazine and loxapine significantly inhibited MAO-A activity and acted as partial inhibitors of MAO-A. Brexpiprazole was found to be the most potent inhibitor (Ľupták, Fišar et al. 2021).

All tested ADs significantly inhibited MAO-A activity, with escitalopram, fluvoxamine and paroxetine acting as full MAO-A inhibitors (Ľupták, Fišar et al. 2022, Ľupták, Fišar et al. 2023).

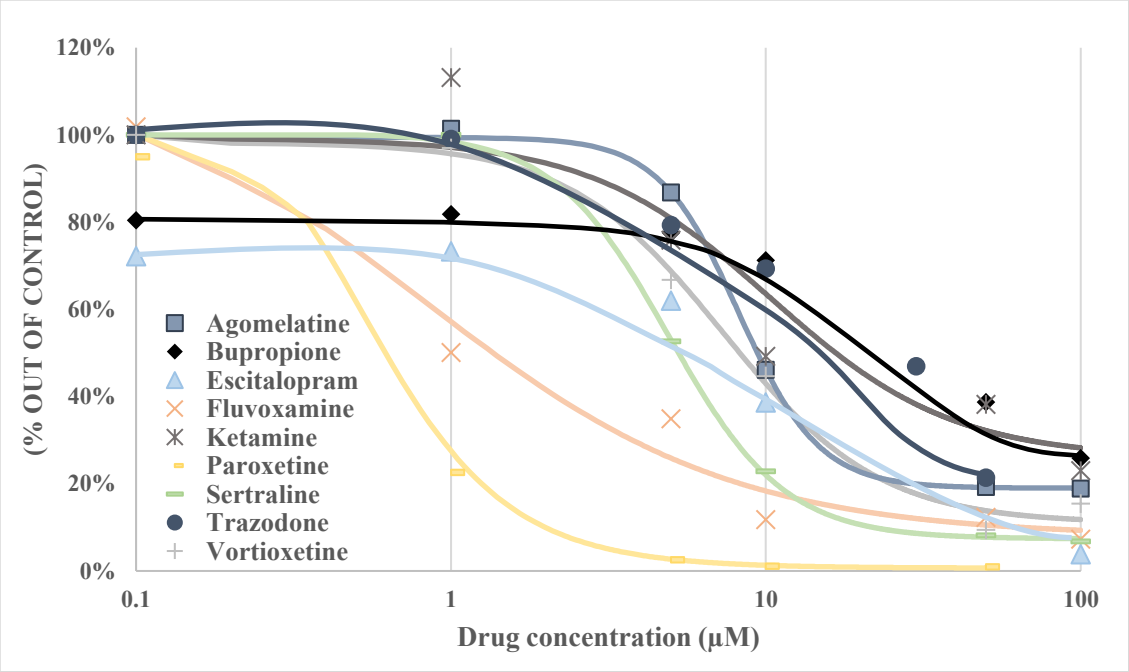
MAO-A inhibition curves are shown in Figure 8A and 8B, and the full list of evaluated parameters is shown in Table 1.

Figure 8A. Antipsychotic-induced inhibition of MAO-A activity



The inhibitory effect of selected antipsychotics on MAO-A activity in a purified fraction of a pig brain mitochondria is shown as dose-response curves. Drug concentrations are plotted in µM on a logarithmic X-axis. Relative activity is expressed as a percentage difference from the activity of the control sample. The lines represent the best-fit curves using a four-parametric logistic function.

Figure 8B. Antidepressant-induced inhibition of MAO-A respiration



The inhibitory effect of selected antidepressants on MAO-A activity in a purified fraction of a pig brain mitochondria is shown as dose-response curves. Drug concentrations are plotted in µM on a logarithmic X-axis. Relative activity is expressed as a percentage difference from the activity of the control sample. The lines represent the best-fit curves using a four-parametric logistic function.

Table 1. Drug-induced inhibition of MAO-A activity

Drug	IC_{50} (µmol/L)	Residual activity (rel. u.)	Hill slope	Inhibition
Antipsychotics				
Brexpiprazole	5.2 ± 8.2	0.33 ± 0.39	0.87 ± 1.20	Partial
Cariprazine	3.2 ± 0.3	0.71 ± 0.07	2.58 ± 8.31	Partial
Loxapine	2.8 ± 3.2	0.71 ± 0.07	1.25 ± 1.65	Partial
Antidepressants				
Agomelatine	8.2 ± 1.4	0.19 ± 0.07	3.40 ± 1.72	Partial
Bupropion	20.2 ± 5.7	0.24 ± 0.05	1.36 ± 0.33	Partial
Escitalopram	8.8 ± 2.3	0.01 ± 0.06	0.99 ± 0.29	Full
Fluvoxamine	1.0 ± 0.2	0.08 ± 0.04	0.91 ± 0.17	Full
Ketamine	10.4 ± 8.3	0.26 ± 0.21	1.50 ± 1.83	Partial
Paroxetine	0.5 ± 0.1	0.01 ± 0.02	1.65 ± 0.22	Full
Sertraline	4.91 ± 0.23	0.07 ± 0.02	1.65 ± 0.22	Full
Trazodone	13.2 ± 2.6	0.18 ± 0.04	1.23 ± 0.22	Partial
Vortioxetine	7.3 ± 1.1	0.11 ± 0.05	1.76 ± 0.61	Partial

IC_{50} is half maximal inhibitory concentration

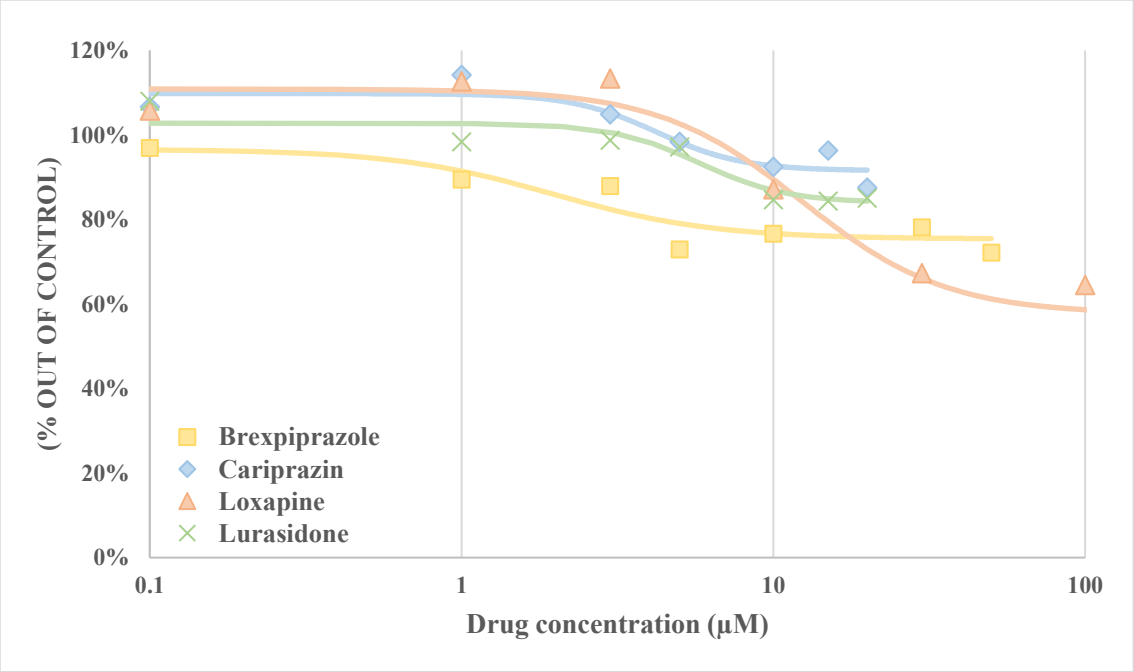
4.5.2. MAO-B activity

MAO-B was inhibited by all tested APs, with brexpiprazole and loxapine being partial MAO-B inhibitors (Ľupták, Fišar et al. 2021).

All tested ADs significantly decreased MAO-B activity, with escitalopram and paroxetine being full MAO-B inhibitors (Ľupták, Fišar et al. 2022, Ľupták, Fišar et al. 2023).

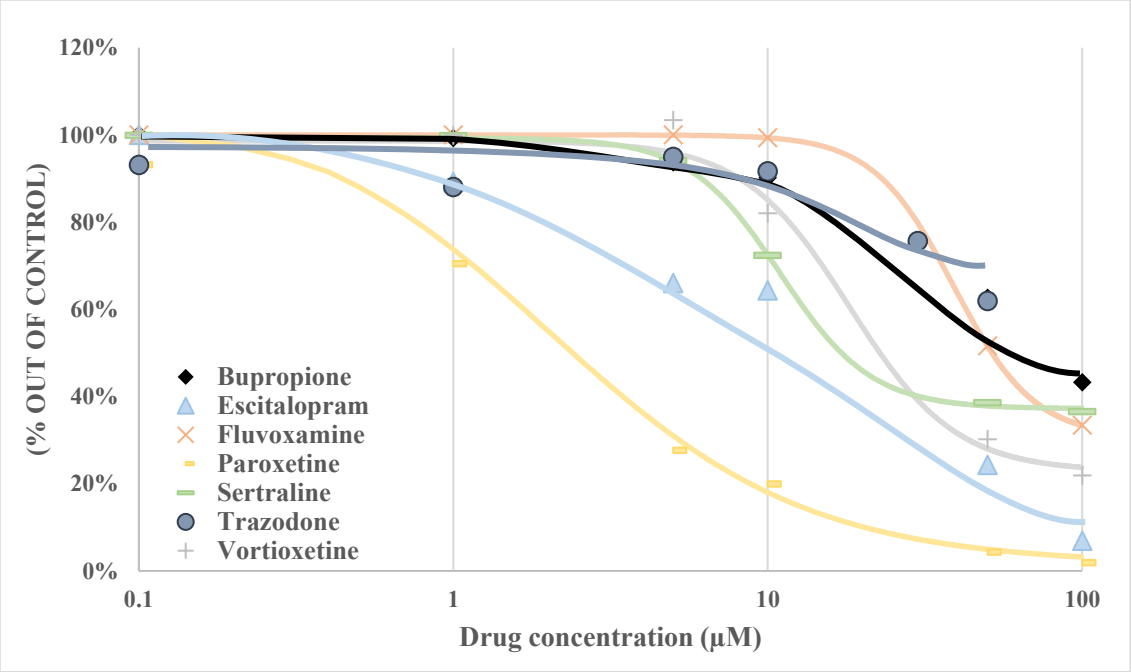
MAO-B inhibition curves are shown in Figure 9A and 9B, and the full list of evaluated parameters is shown in Table 2.

Figure 9A. Antipsychotic-induced inhibition of MAO-B activity



The inhibitory effect of selected antipsychotics on MAO-B activity in a purified fraction of a pig brain mitochondria is shown as dose-response curves. Drug concentrations are plotted in µM on a logarithmic X-axis. Relative activity is expressed as a percentage difference from the activity of the control sample. The lines represent the best-fit curves using a four-parametric logistic function.

Figure 9B. Antidepressant-induced inhibition of MAO-B activity



The inhibitory effect of selected antidepressants on MAO-B activity in a purified fraction of a pig brain mitochondria is shown as dose-response curves. Drug concentrations are plotted in µM on a logarithmic X-axis. Relative activity is expressed as a percentage difference from the activity of the control sample. The lines represent the best-fit curves using a four-parametric logistic function.

Table 2. Drug-induced inhibition of MAO-B activity

Drug	IC_{50} (µmol/L)	Residual activity (rel. u.)	Hill slope	Inhibition
Antipsychotics				
Brexpiprazole	2.0 ± 2.0	0.75 ± 0.06	1.68 ± 2.38	Partial
Cariprazine	4.2 ± 2.5	0.92 ± 0.07	3.14 ± 5.82	Weak
Loxapine	12.4 ± 3.8	0.58 ± 0.06	1.88 ± 1.05	Partial
Lurasidone	5.8 ± 2.3	0.84 ± 0.04	3.10 ± 3.32	Weak
Antidepressants				
Bupropion	31.5 ± 3.3	0.41 ± 0.02	1.68 ± 0.20	Partial
Escitalopram	15.2 ± 2.2	0.04 ± 0.03	1.13 ± 0.14	Full
Fluvoxamine	39.0 ± 2.6	0.31 ± 0.03	3.39 ± 1.35	Partial
Ketamine	51.2 ± 25.54	0.71 ± 0.16	2.12 ± 2.04	Weak
Paroxetine	2.2 ± 0.2	0.02 ± 0.02	1.06 ± 0.10	Full
Sertraline	10.6 ± 0.6	0.37 ± 0.01	3.00 ± 0.68	Partial
Trazodone	29.7 ± 5.8	0.56 ± 0.08	2.75 ± 1.35	Partial
Vortioxetine	18.2 ± 3:5	0.23 ± 0.04	2.58 ± 0.86	Partial

IC_{50} is half maximal inhibitory concentration

5. Discussion

Drug-induced changes in mitochondrial function, energy cell metabolism, and MAO-A and MAO-B activities were evaluated in isolated pig brain mitochondria using APs and ADs with different chemical structures and pharmacological mechanisms of action. We hypothesize that any potential mitochondrial dysfunction, whether drug-induced or disease-related, may have a significant impact on neurotransmission, neuroplasticity, and neurodevelopment. Inadequate energy supply, increased oxidative stress, and increased activity of MAO-A and MAO-B may contribute to the development of psychiatric disorders and influence the adverse effects of psychotropic drugs (Jou, Chiu et al. 2009, Hroudová and Fišar 2011, Fišar 2016). Variability in intracellular processes is likely to be involved in interindividual differences in response to antidepressant and antipsychotic treatment or in drug resistance. 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 has been previously established and evaluated for its suitability to study the effects of drugs on mitochondrial functions (Fišar and Hroudová 2016).

5.1. Mitochondrial respiration

Antipsychotics: Most tested APs significantly inhibit complex I activity, which plays a critical role in cellular energy metabolism and oxygen consumption, and it 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, Luethi, Liechti et al. 2017). Complex I inhibition by APs has been associated with the risk of extrapyramidal adverse effects such as tardive dyskinesia or akathisia. Conventional APs generally show greater complex I inhibition compared to atypical APs (Burkhardt, Kelly et al. 1993, Maurer and Möller 1997, Prince, Yassin et al. 1997, Balijepalli, Kenchappa et al. 2001, Modica-Napolitano, Lagace et al. 2003). Studies have reported decreased complex I activity and complex I-linked respiration in schizophrenic patients treated with APs, with atypical APs being associated with a lower incidence of tardive dyskinesia. A positive correlation was

found between complex I inhibition and the frequency of extrapyramidal symptoms in patients treated with APs (Maurer, Zierz et al. 2001, Rosenfeld, Brenner-Lavie et al. 2011, Gubert, Stertz et al. 2013, Rollins, Morgan et al. 2018).

Although complex II is not directly involved in the generation of the proton motive force required for ATP production (Grimm 2013, Iverson 2013), it can still affect OXPHOS. The positive correlation observed between complex II+III activity and mitochondrial oxygen consumption in the presence of brexpiprazole, cariprazine, loxapine, and lurasidone highlights the importance of drug-induced changes in complex II+III activity on OXPHOS. Notably, zotepine and quetiapine are also potent inhibitors of complex II+III and full inhibitors of complex II-linked respiration, whereas olanzapine has no effect on ETC complexes activity or mitochondrial respiratory rate.

Complex IV is considered an endogenous metabolic marker of neuronal activity (Wong-Riley 1989). Chlorpromazine is a potent inhibitor of ETC complexes I and IV, but only partially inhibits mitochondrial respiration at high concentrations. This suggests that the mitochondrial toxicity of chlorpromazine may not be as high as expected based on its effect on individual ETC complexes. In contrast, zotepine shows a strong inhibitory effect on all individual ETC complexes and fully inhibits mitochondrial respiration, indicating a more pronounced influence on mitochondrial function.

Antidepressants: All tested ADs significantly inhibited all ETC complexes activity and complex I-linked respiration, except bupropion and ketamine, which showed very weak or no inhibition of complexes 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 (SSRIs) and vortioxetine showed more potent complex I inhibition compared to other ADs tested, and overall, the most potent inhibitors of ETC complexes and complex I-linked respiration were vortioxetine, sertraline, and paroxetine. Both sertraline and paroxetine have previously demonstrated their inhibitory properties against mitochondrial parameters (Nadanaciva, Bernal et al. 2007, Li, Couch et al. 2012, Hynes, Nadanaciva et al. 2013). However, complex II-linked respiration remained unaffected, indicating that OXPHOS can still perform respiration through complex II. It appears that all of these ADs selectively inhibit complex I-linked respiration without interfering with the function of complex II in mitochondrial respiration. Bupropion was the only tested AD that inhibited complex II-linked respiration. All tested ADs also showed significant inhibition of complex IV. Previous studies have shown that ADs can have both

stimulatory and inhibitory effects on complex IV activity, whereas other psychoactive drugs typically increase its activity (Holper, Ben-Shachar et al. 2019).

These results are consistent with our previous data with tricyclic antidepressants, SSRIs, and ketamine, which showed significant inhibition of mitochondrial respiration and activity of ETC complexes, particularly complexes I and IV, at high drug concentrations (50-1000 μM) (Hroudova and Fisar 2010, Hroudová and Fišar 2012). This consistency in ADs-induced mitochondrial effects suggests that these drugs may induce similar changes in mitochondrial functions. A clinical study found that higher basal complex I and citrate synthase activity, as well as a greater reduction in complex I activity after treatment, were directly associated with a better response to SSRI treatment in individuals with MDD (Fernström, Mellon et al. 2021).

5.2. ATP production

Antipsychotics: All tested APs showed a decrease in ATP production at 100 μM concentration, which may have implications for ATP-dependent cellular processes and neuronal functions, given the importance of ATP in physiological neuronal function, as a coenzyme, and as a signaling molecule involved in the regulation of neuroplasticity (Filiou and Sandi 2019, Chen, Park et al. 2019, Neupane, Bhuju et al. 2019).

Antidepressants: We have showed that different ADs have distinct effects on mitochondrial ATP production and kinetics. Vortioxetine significantly increased complex I-linked ATP content and complex II-linked ATP kinetics, suggesting the presence of adaptive mechanisms to maintain ATP formation despite vortioxetine's inhibitory properties on individual ETC complexes and complex I-linked respiration. The strongest inhibition of complex IV by ketamine resulted in decreased complex II-linked ATP kinetics, potentially leading to an energetic deficit and increased oxidative stress (Weckmann, Deery et al. 2017). Agomelatine exhibited a significant stimulation of ATP kinetics, possibly due to its higher inhibitory activity on complex I and complex I-linked respiration. These findings are consistent with previous reports indicating that different ADs can alter mitochondrial ATP production in different cell types (Dykens, Jamieson et al. 2008, Rana, Nadanaciva et al. 2011, Li, Couch et al. 2012, Gerö, Szoleczky et al. 2013, Swiss, Niles et al. 2013, Rodrigues, Bristot et al. 2015, Luethi, Liechti et al. 2017, Nabekura, Ishikawa et al. 2022). Considering potent drug-related inhibition of complexes I and IV activity, changes in ATP content and kinetics were smaller, suggesting that ATP production is less sensitive to ADs 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. Mitochondria may also possess reserve capacity and compensatory mechanisms within the OXPHOS, which may contribute to the limited impact of ADs on cellular bioenergetics.

5.3. Hydrogen peroxide formation

Antipsychotics: The tested APs did not significantly increase hydrogen peroxide production, except for cariprazine and loxapine at 100 μM concentration.

Antidepressants: No consistent trend of increased hydrogen peroxide production was observed for the tested ADs at increasing concentrations. Agomelatine and vortioxetine showed a significant increase in hydrogen peroxide content at 10 and 50 μM concentrations, but vortioxetine decreased it at 100 μM concentration. ROS in mitochondria are mainly produced by complexes I and III and 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, Sanson and Riva 2020). While direct binding of ADs to ETC complexes can potentially increase ROS production, there is evidence suggesting that ADs, regardless of their mechanism of action, can improve oxidative stress parameters and enhance antioxidant mechanisms in certain subpopulations of patients. Increased levels of oxidative stress have been associated with a poorer response to SSRI treatment (Abdel-Razaq, Kendall et al. 2011, Fernström, Mellon et al. 2021). It is challenging to determine the consequence of ADs-induced changes in ROS levels due to their dual role as signaling molecules and contributors to oxidative damage. The observed slight increase in total hydrogen peroxide production may lead to signaling changes. Further studies are needed to clarify this hypothesis.

5.4. MAO inhibition

Antipsychotics: All tested APs except lurasidone showed partial inhibition of MAO-A activity. It 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 probably carries the majority of the antidepressant effects (Corponi, Fabbri et al. 2019, Fasipe 2019). Some of these APs are approved for the treatment of various depressive conditions, specifically as adjunctive treatments for MDD

(Diefenderfer and Iuppa 2017). Brexpiprazole and loxapine showed partial inhibition of MAO-B, which may have potential beneficial effects in the treatment of neurodegenerative disorders. In addition, systematic reviews have identified neuroprotective properties of lurasidone, with several mechanisms related to mitochondrial functions (Chen and Nasrallah 2019, Özdemir, Alagöz et al. 2021).

Antidepressants: All of the tested ADs showed either full or partial MAO-A inhibition, which may contribute to their antidepressant effect. MAO inhibitors were historically used as the first-generation ADs. Paroxetine and fluvoxamine inhibited MAO-A at therapeutic concentrations (0.1-1 μM). Previous studies have reported MAO-inhibitory properties of these ADs (Bryant, Guernsey et al. 1983, Ferris, Cooper et al. 1983, Gandolfi, Barbaccia et al. 1983, Johnson 1989, Mukherjee and Yang 1997, Mukherjee and Yang 1999, Pivac, Mück-Seler et al. 2003). Vortioxetine, paroxetine, and sertraline exhibited significant inhibition of MAO-B, which could be used therapeutically in neurodegenerative disorders by reducing ROS production during oxidation of monoamine neurotransmitters. Vortioxetine-induced MAO-B inhibition may also contribute to its beneficial effect on cognitive deficits in patients with MDD (Thomas 2000, Frampton 2016). These results suggest that MAO inhibition may play a role in both the antidepressant and neuroprotective effects of the tested ADs, although they are not classified as MAO inhibitors.

5.5. Research limitations

The main focus of this work was to measure the effects of psychotropic drugs on complex mitochondrial parameters (mitochondrial respiration, mitochondrial enzymes and MAO activity, and ATP and ROS production) *in vitro* in isolated mitochondria. This approach allows a more accurate detection of drug effects on mitochondrial compensatory and regulatory mechanisms than is possible with *in vivo* measurements. The regulatory and compensatory brain mechanisms that may influence the effects of tested drugs on mitochondrial functions *in vivo* in our experimental approach (e.g., effects of drugs on mitochondrial dynamics, fusion, and fission) are not covered by our experimental approach.

Measurement methods and the intricate relationship between drug effects on individual ETC complexes and overall mitochondrial functions add to the complexity of understanding mitochondrial toxicity. Different drugs may also induce compensatory or neuroprotective mechanisms that can mitigate mitochondrial dysfunction, highlighting

the potential modulation of other mitochondrial proteins beyond ETC complexes by psychopharmaca.

Due to the lipophilic nature and the presumed accumulation of psychopharmaca in neuronal membranes and subcellular structures, including mitochondria, higher concentrations of these drugs were used in the experiments compared to their therapeutic plasma concentrations. Although caution must be exercised in extrapolating these findings from experimental studies to clinical situations, preliminary evidence suggests that psychopharmaca may affect mitochondrial functions and may induce adverse effects at higher concentrations.

5.6. Possible clinical impact

Antipsychotics: APs were associated with adverse effects related to mitochondrial dysfunction. Complex I inhibition has been associated with extrapyramidal symptoms as well as QTc interval prolongation (Glassman and Bigger 2001, Haddad and Anderson 2002, Leucht, Cipriani et al. 2013). Treatment with second-generation APs is associated with an increased risk of cardiovascular events and metabolic syndrome, which may be related to alterations in mitochondrial homeostasis (Del Campo, Bustos et al. 2018). While APs 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 APs on mitochondrial respiration. Nevertheless, some APs may exhibit additive antidepressant or neuroprotective effects, or induce adaptive processes that compensate for mitochondrial toxicity, highlighting the intricate balance between therapeutic and side effects of APs.

Antidepressants: Our research suggests that pharmacologically distinct ADs 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 ADs may be related to the adverse effects associated with AD therapy. Vortioxetine, sertraline and paroxetine, exert very potent inhibitory properties against ETC complexes and mitochondrial respiratory rate, which may contribute to their adverse effects. Although generally safe and well tolerated, vortioxetine has been associated with higher treatment discontinuation rates than placebo, and its increasing doses may worsen tolerability (Kelliny, Croarkin et al. 2015, He, Wang et al. 2018, Gonda, Sharma et al. 2019). Sertraline and paroxetine are known to have lower

tolerability profile among SSRIs, which may be related to mitochondrial toxicity, specifically complex II+III inhibition (Westenberg and Sandner 2006, Mandrioli, Mercolini et al. 2012, Sanchez, Reines et al. 2014). 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 ADs may serve as a starting point for complex cellular responses within the intracellular environment, ultimately leading to adaptive changes that support neuroplasticity. We can hypothesize that mild antimitochondrial effects of ADs may provide a potentially protective preconditioning effect. This suggests that ADs-induced mitochondrial dysfunction, below the threshold of damage, may subsequently provide protection or enhance neuroplasticity (Calabrese, Cornelius et al. 2010, Abdel-Razaq, Kendall et al. 2011). Some ADs have shown modest beneficial effects on cognitive function in depressed patients, which may be related to MAO inhibition, demonstrating procognitive effects or potential prevention of cognitive decline with long-term use (Bartels, Wagner et al. 2018, Prado, Watt et al. 2018, Blumberg, Vaccarino et al. 2020, Gonçalo and Vieira-Coelho 2021). However, the effects of ADs on cognition are complex, involve multiple mechanisms, and vary by drug and patient population.

6. Conclusion

In vitro studies of APs and ADs reveal important and statistically significant drug-induced changes in selected mitochondrial parameters, especially at high concentrations. The results can be summarized as follows:

- complex I, complex IV, and complex I-linked respiration were the most affected parameters
- 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 adverse effects of ADs and APs
- activation of mitochondrial compensatory mechanisms and MAO inhibition may contribute to therapeutic effects of ADs and APs
- there is a relationship between the activity of individual ETC complexes, they may affect each other
- complex I-linked respiration is more dependent on activities of ETC complexes than complex II-linked respiration
- although there is a strong correlation between the activity of individual ETC complexes and complex I-linked respiration, this correlation is not unequivocal
- the precise molecular mechanisms by which various psychopharmaca affect other mitochondrial function remain to be determined in further clinical research

7. References

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8. List of attachments

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