In vitro inhibition of mitochondrial respiratory rate by antidepressants

Jana Hroudová, Zdeněk Fišar*
Department of Psychiatry, First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague, Ke Karlovu 11, 120 00 Prague, Czech Republic

**HIGHLIGHTS**
- Effects of psychotropic drugs on respiratory rate were measured in brain mitochondria.
- Inhibitory effects of tianeptine, fluoxetine, amitriptyline and chlorpromazine were found.
- Antidepressants, but not mood stabilizers, are potent partial inhibitors of respiration.

**ABSTRACT**
Mitochondria represent a possible drug target with unexplored therapeutic and toxicological potential. The possibility was suggested that antidepressants, mood stabilizers and other drugs may show some therapeutic and/or toxic effects through their action on mitochondrial functions. There are no sufficient data about the effect of these drugs on mitochondrial respiration in the brain. We investigated the in vitro effects of amitriptyline, fluoxetine, tianeptine, ketamine, lithium, valproate, olanzapine, chlorpromazine and propranolol on mitochondrial respiration in crude mitochondrial fractions of pig brains. Respiration was energized using substrates of complex I or complex II and dose dependent drug-induced changes in mitochondrial respiratory rate were measured by high-resolution respirometry. Antidepressants, but not mood stabilizers, ketamine and propranolol were found to inhibit mitochondrial respiratory rate. The effective dose of antidepressants reaching half the maximal respiratory rate was in the range of 0.07–0.46 mmol/L. Partial inhibition was found for all inhibitors. Differences between individual drugs with similar physicochemical properties indicate selectivity of drug-induced changes in mitochondrial respiratory rate. Our findings suggest that mood stabilizers do not interfere with brain mitochondrial respiration, whereas direct mitochondrial targeting is involved in mechanisms of action of pharmacologically different antidepressants.

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1. Introduction
Impaired function of mitochondria leads to impaired bioenergetics, decreased ATP production, impaired calcium homeostasis, increased production of free radicals and oxidative stress and to initiation of apoptotic processes (Fišar and Hroudová, 2010; Hroudová and Fišar, 2011). There is mounting evidence for the role of mitochondrial dysfunction in the pathophysiology and treatment of neurodegenerative diseases, including mood disorders (Kato and Kato, 2000; Stork and Renshaw, 2005). Thus, mitochondria are now the target for therapeutic interventions and enhancement of mitochondrial function may represent a critical component for the optimal treatment of neurodegenerative and stress-related diseases (Quiroz et al., 2008; Frantz and Wipf, 2010; Schapira, 2012). In contrast, drug-induced disruption of mitochondrial oxidative phosphorylation is a mechanism of toxicity, which disturbs mitochondrial respiration and thereby alters energy metabolism.

At the mitochondrial level, there are several potential drug targets that can lead to toxicity. Thus, it is important to test for mitochondrial toxicity in the early phase of new drug development, as impairment of mitochondrial function can induce various pathological conditions or can increase progression of existing diseases (Chan et al., 2005; Boelsterli and Lim, 2007; Fišar et al., 2010, 2011; Scatena, 2012). E.g., valproic acid and nefazodone as central nervous system drugs showed both mitochondrial liability and potential hepatic and cardiovascular toxicity (Dykens and Will, 2007; Boelsterli and Lim, 2007).

Although a wide range of pharmacologically different antidepressants and mood stabilizers is available, molecular mechanisms of their therapeutic or side effects have not yet been sufficiently clarified. There is little information about the association between the therapeutic and/or adverse effects of these drugs...
and mitochondrial respiration. Assessment of mitochondrial dysfunctions and drug cytotoxicity may be important in drug development as well as in the study of drug interactions (Dykens et al., 2008). The hypothesis that antipsychotic-induced extrapyramidal side effects may be due to inhibition of the mitochondrial respiratory chain was reported previously (Maurer and Möller, 1997).

1040 drugs and other bioactive compounds were tested as potential inhibitors of mitochondrial permeability transition in rat liver mitochondria (Stavrovskaya et al., 2004). It was found that heterocyclics, including antipsychotics, antidepressants, antihistaminics and others mediate dose-dependent protection that is not related to therapeutic class. Recently, the effects of selected antidepressants (clomipramine, desipramine, norfluoxetine, tianeptine) on mitochondrial functions such as caspase-3 activity, membrane potential, mitochondrial electron transport chain complex activities and mitochondrial oxygen consumption were investigated in a model cell system or in isolated mitochondria (Abdel-Razaq et al., 2011). The data suggest that complex I may be more sensitive to antidepressant inhibition than other complexes of the electron transport chain (ETC).

More than 60 different types of compounds, including antidepressants and mood stabilizers (Hroudova and Fisar, 2010), are well-known inhibitors of complex I. Due to its complexity, complex I is vulnerable to lipophilic molecules. Complex II has been less studied in mitochondrial pharmacotoxicology. For complex III, there are a large number of inhibitors with no known clinical value. There are a number of well-known entities that inhibit complex IV. Moreover, some polycationic molecules can interact with mobile electron carriers.

There are several interconnections and feedbacks within the system of oxidative phosphorylation (OXPHOS). Thus, inhibition of the activation of one component (e.g. complex I or complex IV) does not provide any information about the final effect of a drug on cellular respiration and energetics. The bioenergetics function of mitochondria can be investigated by measuring the rate of ATP formation and the efficiency of the process (the P/O ratio, i.e. the ratio of ATP formed over oxygen consumed) (Merlo-Pich et al., 2004). Measurement of oxygen consumption and its sensitivity to substrates, uncouplers and inhibitors can be a good indicator of mitochondrial phosphorylative capacity. To provide a routine approach to the study of oxygen kinetics, multiple substrate-uncoupler-inhibitor titration protocols for high-resolution respirometry were developed for the accurate measurement using small amounts of tissue, cells and isolated mitochondria (Pesta and Gnaiger, 2012).

Respiratory rate is a parameter characterizing functioning of the OXPHOS system as a whole. Drug effect on respiratory rate was measured in the work presented here using pharmacologically different psychotropic drugs known to affect specific mitochondrial functions: 1. amitriptyline, fluoxetine and tianeptine (antidepressants); 2. ketamine (glutamate N-methyl-D-aspartate receptor antagonist; anesthetic and potential antidepressant) (Rezin et al., 2009; de Oliveira et al., 2009); 3. lithium and valproate (mood stabilizers) (Haas et al., 1981; Ponchait et al., 1992; Silva et al., 1997; Bachmann et al., 2009; Maurer et al., 2009); 4. olanzapine (atypical antipsychotic and mood stabilizer) (Lauterbach et al., 2010); 5. chlorpromazine (atypical antipsychotic); and 6. propranolol (non-selective beta blocker) (Katyare and Rajan, 1991; Robinson et al., 2009; Kaasik et al., 2010; Wills et al., 2012). Our study was aimed at discovering the effects of antidepressants and mood stabilizers on mitochondrial respiratory rate. The effect of chlorpromazine was measured for comparison, as its mitochondrial effects are well-known. Propranolol, a drug which can induce depressive symptoms, was tested to compare the effect of a molecule with similar physicochemical properties as antidepressants, however with a diametrically different pharmacological mode of action.

Amitriptyline is a tricyclic antidepressant, whose primary biochemical action related to its therapeutic effects is serotonin and norepinephrine reuptake inhibition. Its adverse effects are due to the antagonism of α1-adrenergic, histamine and muscarinic acetylcholine receptors. Recently, amitriptyline was found to be an inhibitor of mitochondrial functions, e.g. mitochondrial membrane permeability transition (Stavrovskaya et al., 2004). Acute in vivo administration of amitriptyline caused a general pattern of decrease in brain oxidative metabolism (González-Pardo et al., 2008). Amitriptyline treatment induced oxidative stress in several tissues, including brain, in a dose-dependent manner. However, coenzyme Q was increased in the brain after long-term treatment, probably in order to counteract oxidative damage (Bautista-Ferrufino et al., 2011).

Fluoxetine together with its active metabolite norfluoxetine is an antidepressant of the selective serotonin reuptake inhibitor (SSRI) class. Its adverse side effects are less pronounced compared to amitriptyline and include sexual dysfunctions, nausea, insomnia, somnolence, anorexia, anxiety, nervousness, asthenia and tremor. Fluoxetine and norfluoxetine inhibit many isoenzymes of the cytochrome P450 system that make drug metabolism possible. Furthermore, they inhibit the activity of P-glycoprotein that plays an important role in drug transport and metabolism. The simultaneous use of fluoxetine with serotonergic agents can result in a serotonin syndrome. Fluoxetine has multiple effects on the energy metabolism of rat liver mitochondria, being potentially toxic in high doses (Souza et al., 1994). It affects apoptosis through an increase of the voltage sensitivity of the mitochondrial voltage-dependent anion channel (Nahon et al., 2005). Fluoxetine induces inhibition of OXPHOS and decreases the activity of ATP synthase in rat brain mitochondria (Curti et al., 1999). Apoptotic effects, reduction of membrane potential, inhibition of the activity of mitochondrial complexes and decrease of state 3 respiration were observed after exposure to norfluoxetine (Abdel-Razaq et al., 2011).

Tianeptine is an antidepressant drug, which was first classified as a selective serotonin reuptake enhancer. However, the more recent view is that the therapeutic mechanism of action of tianeptine can be attributed to its effect on the glutamatergic system and the reversal of impaired neuroplasticity associated with stress (McEwen et al., 2010). Its side effects are less pronounced compared to amitriptyline and include dry mouth, constipation, dizziness, drowsiness, postural hypotension, insomnia and vivid dreams, headaches and hypomania induction. Tianeptine inhibits complex I activity; other mitochondrial complexes, apoptotic effects and reduction of membrane potentials were not affected following exposure of model cell systems or isolated mitochondria to tianeptine (Abdel-Razaq et al., 2011).

Chlorpromazine is a typical antipsychotic. It is a very effective antagonist of D2-type dopamine receptors, but also produces anticholinergic, antihistaminic and weak antidiurenergic effects. It is known as an inhibitor of mitochondrial functions, e.g. complex I (Modica-Napolitano et al., 2003), mitochondrial nitric oxide synthase (Lores-Arnaiz et al., 2004), mitochondrial phospholipase A2 and mitochondrial membrane permeability transition (Furuno et al., 2001; Stavrovskaya et al., 2004).

Common physicochemical properties of drugs may be associated with their similar effect on certain cellular functions. Cationic amphiphilic drugs include antidepressants, antipsychotics, calcium channel blockers, beta receptor blockers, antihistaminics, and antifungals. The technically incorrect term “cationic amphiphilic drug” comprises drug compounds that have a hydrophobic part consisting of a nonpolar ring part and a hydrophilic group.
with one or more nitrogen containing groups, which can bear a net positive charge at physiological pH. Cationic amphiphilic drugs may induce phospholipidosis (Xia et al., 2000; Reasor and Kacew, 2001). However, the relationship between drug-induced phospholipidosis and adverse drug effects remains unexplained (Anderson and Borlak, 2006).

Drug lipophilicity, charge, polar surface area and membrane potential influence mitochondrial drug delivery, with the uptake of positively charged, lipophilic molecules being the most efficient (Durazo et al., 2011). From a physicochemical point of view, amitryptiline, fluoxetine, tianeptine, ketamine, olanzapine, chlorpromazine and propranolol are all cationic amphiphilic drugs, which can accumulate in mitochondria.

The mitochondrial hypothesis states that impaired energy metabolism of brain cells is involved in the pathophysiology of mood disorders and in the effects of antidepressants and mood stabilizers. We presume that the therapeutic or side effects of drugs administered in the treatment of depression may involve the targeted regulation of mitochondrial functions and the subsequent effect on neuroplasticity, disease related inflammatory responses, calcium homeostasis, production of reactive oxygen and nitrogen species and other processes related to the complex response to stress, neurotoxicity or impaired neurotransmission. On the basis of this hypothesis, we studied the effects of antidepressants and mood stabilizers on specific enzymes and complexes of the respiratory chain (Fišar et al., 2010; Hroudova and Fišar, 2010). In the study presented here, we measured the effects of these drugs on the respiratory rate of intact mitochondria, i.e. we measured drug-induced changes on mitochondrial function as a whole. We considered functional parameters such as respiratory rate, mitochondrial permeability transition or inner membrane potential (i.e. measurements that reflect activity of the intact respiratory system) to be more suitable for evaluating drug effects on cellular energetics than the measurement of specific mitochondrial enzymes. Our study has the potential to contribute to a better understanding of the role of mitochondria in the mechanism of action of antidepressants and to evaluate positive and negative effects of the tested drugs on brain bioenergetics.

There is a need both for predictive and retrospective in vitro assays of drug-induced mitochondrial toxicity. A large number of drugs that have been withdrawn from the market or stopped during development due to hepatotoxicity, nephrotoxicity or cardiotoxicity have been reported to disturb mitochondrial functions. The aim of the present study was to investigate the effects of antidepressants and mood stabilizers of different chemical structures on mitochondrial respiratory rate in intact brain mitochondria. In vitro effects of pharmacologically different antidepressants (amitryptiline, fluoxetine, tianeptine), mood stabilizers (lithium, valproate, olanzapine) and other drugs (ketamine, chlorpromazine, propranolol) on the mitochondrial respiratory rate were measured in crude mitochondrial fractions isolated from pig brain using high-resolution respirometry. Pig mitochondria are relatively often used in studies of mitochondrial functions and enzymes. Moreover, the pig model is commonly used in immunological studies due to the similarity of the human and pig immune system, and more precise measurement in the brain structures is possible in pig brains compared to rat or mice brains. Respiration was characterized by respiratory state 3 (Chance and Williams, 1955; Gnaiger, 2009) using substrates for electron supply either through complex I (malate- and pyruvate-energized mitochondria) or through complex II (succinate-energized mitochondria) of ETC. Extended-range titration of mitochondrial suspensions by drugs enables the determination of half maximal inhibitory concentrations and other inhibitory parameters applicable for evaluating the potential mitochondrial toxicity of individual drugs.

2. Materials and methods

2.1. Materials

The mitochondrial respiration medium (MIR05) consisted of sucrose 110 mmol/L, K-lactobionate 60 mmol/L, tauroine 20 mmol/L, MgCl₂ 1.89 mmol/L, KH₂PO₄, pO₄Na₂ (0.5 mmol/L, BSA 1 g/L, HEPES 20 mmol/L, adjusted to pH 7.1 with KOH (Kuznetsova et al., 2004; Pesta and Gnaiger, 2012). Substrates, inhibitors or drugs were added to samples containing mitochondria as described in the protocols below. Hamilton syringes were used for manual titration and the automatic titration-injection micropump TIP2k (Orbros Instruments, Innsbruck, Austria) was used for drug titration. The effects of various antidepressants (amitryptiline, fluoxetine, tianeptine, ketamine) and mood stabilizers (lithium carbonate, sodium valproate, olanzapine) were tested. The effects of chlorpromazine and propranolol were measured for comparison. The chemicals were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA), except for fluoxetine, tianeptine and olanzapine which were a gift from Zentiva Group a.s. (Prague, Czech Republic).

2.2. Animal brain mitochondria

The mitochondria were isolated from the pig brain cortex using homogenization and differential centrifugation as previously described (Fišar et al., 2010). In brief, gray matter was homogenized in ice-cold buffered sucrose (0.32 mol/L sucrose, 4 mmol/L HEPES; pH 7.4). Tissue homogenate was centrifuged at 1000 × g for 10 min and the supernatant was carefully decanted. The pellet was resuspended in buffered sucrose and centrifuged again under the same conditions. Supernatants were collected and recentrifuged at 10,000 × g for 15 min. The final pellet containing the crude mitochondrial fraction (including synaptosomes) was rinsed twice with buffered sucrose (10,000 × g, 15 min), resuspended to a protein concentration of 20–40 mg/mL and preserved in cold storage at 0–4 °C. Protein concentration was determined using the method of Lowry et al. (1951), with bovine serum albumin as the standard.

2.3. High-resolution respirometry

Mitochondrial respiratory system activity was measured at 37 °C in a titration-injection high-resolution oxygen graph (Oxygraph-2k, Orbros Instruments, Innsbruck, Austria) equipped with two glass-clay respiration chambers with Clark-type polarographic oxygen electrodes. The sample was placed into the incubation chamber and stirred continuously using a magnetic stirring bar. High-resolution respirometry is based on sensitive monitoring of oxygen concentration in the sample over time and calculation of the rate of oxygen consumption. Data were collected and analyzed using the DatLab 4.3 software (Orbros Instruments, Innsbruck, Austria) displaying the real-time oxygen concentration and oxygen flux, which is the negative time derivative of oxygen concentration. Oxygen solubility factor relative to distilled water for MIR05 was set at 0.92 (Sjøvold et al., 2010). Respiratory rates (oxygen fluxes) were expressed as pmolO₂ consumed per second relative to one milligram of protein in the sample of crude brain mitochondria (pmolO₂/(mg s)).

2.4. Drug effects on the respiration rate of brain mitochondria

Isolated pig brain mitochondria were used as an in vitro model to study the effects of antidepressants and mood stabilizers on mitochondrial oxygen consumption rates. Digitonin was used to permeabilize the plasma membrane of synaptosomes present in the crude mitochondrial fraction. Digitonin permeabilizes plasma membranes, whereas mitochondrial membranes are affected only at higher concentrations. To differentiate the effect of drugs on the respiratory capacities achieved through complex I and complex II, separate respirometric measurements for complex I as well as complex II were performed. Thus, two multiple substrate-inhibitor titration protocols were used to determine the changes in respiration rate induced by different concentrations of amitryptiline, fluoxetine, tianeptine, ketamine, lithium, valproate, olanzapine, and chlorpromazine. At least 17-point titration was performed, so that final drug concentrations were in the range 0.5–2500 µmol/L. Because one titration experiment takes more than 1 h and the respiratory rate of samples in closed chamber significantly decreases during this period, two parallel samples were titrated and measured simultaneously. The sample in the first chamber was titrated by the drug and the sample in the second chamber was titrated by the medium used as the drug solvent.

2.0 µL MIR05 in measuring chambers were saturated with atmospheric oxygen for about 30 min. The chambers were closed and 2–10 µL of crude mitochondria were injected so that the resulting protein concentration in the sample was between 50 and 200 µg/mL. 10 µL of digitonin (50 µg/mL) were added immediately to permeabilize the synaptosomes.

(a) Respiration through complex I was initiated by adding 2 µmol/L malate and 5 mmol/L pyruvate. Following signal stabilization, state 3 respiration was induced by the addition of 1.25 mmol/L adenosine diphosphate (ADP) and titration with 0.5–100 µL drug/medium was carried out using TIP2k with 3 min intervals between single titrations. Finally, complex I was blocked by 0.5 µmol/L rotenone and residual
Table 1  Drug-induced inhibition of respiratory rate in pig brain mitochondria incubated with substrates for electron supply through complex I.

<table>
<thead>
<tr>
<th>Drug</th>
<th>IC50 (μM/L)</th>
<th>Hill slope</th>
<th>N</th>
<th>Residual (rel.u.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amitriptyline</td>
<td>176.2 ± 9.4</td>
<td>1.70 ± 0.12</td>
<td>10</td>
<td>0.714</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>86.2 ± 0.5</td>
<td>1.53 ± 0.19</td>
<td>8</td>
<td>0.562</td>
</tr>
<tr>
<td>Tianeptine</td>
<td>88.9 ± 2.6</td>
<td>2.95 ± 0.23</td>
<td>9</td>
<td>0.739</td>
</tr>
<tr>
<td>Ketamine</td>
<td>361.6 ± 21.5</td>
<td>3.70 ± 0.70</td>
<td>5</td>
<td>0.886</td>
</tr>
<tr>
<td>Lithium</td>
<td>nd</td>
<td>nd</td>
<td>8</td>
<td>1.000</td>
</tr>
<tr>
<td>Valproate</td>
<td>nd</td>
<td>nd</td>
<td>7</td>
<td>0.964</td>
</tr>
<tr>
<td>Olanzapine</td>
<td>nd</td>
<td>nd</td>
<td>9</td>
<td>0.964</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>115.9 ± 10.7</td>
<td>1.67 ± 0.19</td>
<td>8</td>
<td>0.530</td>
</tr>
<tr>
<td>Propranolol</td>
<td>107.4 ± 38.9</td>
<td>2.31 ± 1.44</td>
<td>6</td>
<td>0.921</td>
</tr>
</tbody>
</table>

Values are means ± standard deviation; IC50, half maximal inhibitory concentration; N, number of measurement; Residual, residual activity at high drug concentration.

Table 2  Drug-induced inhibition of respiratory rate in pig brain mitochondria incubated with substrates for electron supply through complex II.

<table>
<thead>
<tr>
<th>Drug</th>
<th>IC50 (μM/L)</th>
<th>Hill slope</th>
<th>N</th>
<th>Residual (rel.u.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amitriptyline</td>
<td>462.6 ± 25.5</td>
<td>2.58 ± 0.22</td>
<td>10</td>
<td>0.662</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>266.2 ± 8.9</td>
<td>3.37 ± 0.30</td>
<td>9</td>
<td>0.540</td>
</tr>
<tr>
<td>Tianeptine</td>
<td>67.4 ± 4.9</td>
<td>2.39 ± 0.36</td>
<td>8</td>
<td>0.717</td>
</tr>
<tr>
<td>Ketamine</td>
<td>nd</td>
<td>nd</td>
<td>4</td>
<td>1.000</td>
</tr>
<tr>
<td>Lithium</td>
<td>nd</td>
<td>nd</td>
<td>8</td>
<td>1.000</td>
</tr>
<tr>
<td>Valproate</td>
<td>nd</td>
<td>nd</td>
<td>7</td>
<td>0.956</td>
</tr>
<tr>
<td>Olanzapine</td>
<td>418.6 ± 213.6</td>
<td>2.16 ± 0.77</td>
<td>7</td>
<td>0.878</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>262.6 ± 33.0</td>
<td>2.38 ± 0.44</td>
<td>10</td>
<td>0.395</td>
</tr>
<tr>
<td>Propranolol</td>
<td>395.4 ± 332.7</td>
<td>1.34 ± 0.78</td>
<td>5</td>
<td>0.918</td>
</tr>
</tbody>
</table>

Values are means ± standard deviation; IC50, half maximal inhibitory concentration; N, number of measurement; Residual, residual activity at high drug concentration.

Oxygen consumption was measured to be subtracted from all the other respiratory rate values.

(b) Activation of respiration through complex II was preceded by the addition of 1.25 mM/L ADP and by complex I inhibition using 0.5 μM/L rotenone. State 3/4 respiration was then induced by the addition of 10 mM/L succinate and titration with 0.5–100 μL drug/medium was carried out using TPP2 with 3 min intervals between single titrations. Finally, respiration was blocked by 1.25 μg/mL antimycin A and residual oxygen consumption was measured to be subtracted from the other respiratory rate values.

Following subtraction of residual oxygen consumption from respiratory rates at different drug/medium concentrations, differences among drug-treated and medium-titrated samples were calculated and relative drug-induced changes in respiratory rate were determined, presuming that the relative respiratory rate equals to 1 at zero drug concentration.

2.5. Data analysis

All data presented are expressed as the mean ± standard deviation. Results were analyzed using STATISTICA (data analysis software system, version 10.0, StatSoft, Inc., Tulsa, OK, USA). The differences in means between two groups were evaluated using the Mann–Whitney U test. Inhibition of respiratory rate by drugs was analyzed using the four-parameter logistic function (SigmaPlot, Systat Software, Inc., Richmond, CA, USA) to establish the concentration of a drug that is required for 50% inhibition in vitro (IC50), Hill slope, which quantifies the steepness of a dose–response curve at its midpoint was used to determine the degree of cooperativity of the ligand binding to the enzyme and residual activity at high drug concentration (Residual):

\[
y = \min + \frac{(\max - \min)}{1 + \left(\frac{x}{IC50_{\text{Hill slope}}}\right)^{Hill \ slope}} \quad \text{Residual} + \frac{(\max - \text{Residual})}{1 + \left(\frac{x}{IC50}\right)^{Hill \ slope}}
\]

where x is drug (inhibitor) concentration and y is relative respiratory rate (equal to 1 free from inhibitor).

3. Results

The effects of antidepressants (amitriptyline, fluoxetine, tianeptine, and ketamine), mood stabilizers (lithium carbonate, sodium valproate, and olanzapine), propranolol and chlorpromazine on respiration rate in pig brain mitochondria were assessed and compared with the effect of chlorpromazine. Inhibitory parameters were determined by analyzing dose–response curves of the mitochondrial respiratory rate in relation to the drug concentration.

Data are shown in Figs. 1 and 2 and the parameters characterizing the potency of tested drugs in inhibiting mitochondrial respiratory rate are summarized in Tables 1 and 2. As expected, chlorpromazine was a potent inhibitor (with relatively low residual activity at high drug concentrations) of respiration energized using substrates of complex I as well as substrates of complex II. Tianeptine showed the highest efficacy of inhibition among the tested antidepressants. Fluoxetine inhibited respiration through complex I with higher potency compared to inhibition of respiration through complex II. Amitriptyline was a less potent inhibitor of respiratory rate compared to both tianeptine and fluoxetine. Ketamine was a weak inhibitor (with high residual activity) of respiration through complex I and respiration through complex II was not inhibited by ketamine. In contrast, olanzapine was a weak inhibitor of respiration through complex II only. Lithium and valproate did not affect respiratory rate, even at very high concentrations. Inhibition by propranolol was very low with residual activity as much as 92%.

The Hill slope of all inhibitory curves was significantly higher than 1, indicating a positive combination of the drug and residual activity was observed for all inhibiting drugs, including chlorpromazine (Tables 1 and 2, Figs. 1 and 2) indicating that the tested drugs are either none or partial inhibitors of mitochondrial respiratory rate.

4. Discussion

The action of various therapeutically applied drugs on mitochondria is relatively unknown. Some drugs have been specifically designed to affect mitochondrial functions. However, most of them primary act on other cellular targets and may modify mitochondrial functions as adverse effects (Szewczyk and Wojtczak, 2002). Our study demonstrated that pharmacologically different antidepressants, but not mood stabilizers can inhibit respiratory rate in mitochondrial preparations from brain tissue. The experimental conditions of the present study, use of the selective substrates malate and pyruvate for complex I and succinate for complex II, allowed the separate evaluation of drug effects on respiration activated through complex I and through complex II. We tested the hypothesis that pharmacologically different antidepressants or mood stabilizers could act, at least in part, via changes in brain cell energetics. Thanks to the wide-range of drug concentrations in our titration experiments, we were able to calculate matching inhibitory parameters, useful for the quantitative comparison of the effects of different drugs on mitochondrial respiratory rate.

We confirmed and extended previous results relating to the inhibitory effects of antidepressants of various pharmacological classes on OXPHOS. All tested drugs, except for tianeptine and olanzapine, were more potent inhibitors of respiration through complex I than of respiration through complex II. Fluoxetine, as well as amitriptyline, appear to be selective inhibitors of respiratory rate through complex I. Tianeptine was found to be a potent inhibitor of mitochondrial respiration energized through both complexes. In contrast, ketamine inhibited mitochondrial respiration very weakly or not at all, which can be indicative of its different mechanism of action. Significant differences between several tested antidepressants indicate the existence of certain selectivity of antidepressant–mitochondria interactions, regardless of the similar physicochemical properties of their molecules. All tested mood stabilizers showed only a minimal effect on respiratory rate and it may be assumed that direct mitochondrial targeting is not part of their effect.

In the present study, all drugs tested inhibited mitochondrial respiration only at higher concentrations. These concentrations were much higher than therapeutically active plasma.
concentrations of antidepressants in vivo. However, most of antidepressants are cationic amphiphilic molecules, which can accumulate in the brain, membranes and subcellular components (Caccia et al., 1990; Karson et al., 1993; Fišar et al., 1996, 2004; Fišar, 2005). The unique physicochemical properties of the mitochondrial matrix may facilitate the selective accumulation of different xenobiotics in the matrix and/or in the inner mitochondrial membrane (Szewczyk and Wojtczak, 2002). In the presence of membrane potential, the distribution of charged lipophilic cations across the membrane will equilibrate with the membrane potential leading to the extensive accumulation of the cation in the mitochondrial matrix. There will be a 10-fold accumulation of the cation within mitochondria for every 61.5 mV (at 37 °C) increase in membrane potential. As mitochondrial ΔΨm is typically 140–180 mV, there will be a several hundred-fold accumulation of lipophilic cations in the mitochondrial matrix (Ross et al., 2006). However, according to a review of literature, there is no simple correlation between the mitochondria targeting capacity and physicochemical properties of drugs. It was concluded that the most common physicochemical factors underlying selective accumulation of xenobiotics within mitochondria are electric potential, ion-trapping and complex formation with cardiolipin; nonspecific accumulation involves membrane partitioning (Horobin et al., 2007). Thus, high antidepressant concentrations can be expected in mitochondria, sufficient for partial inhibition of the respiratory rate.

It cannot be ignored that metabolism and transport of drugs are ATP consuming processes. Thus, inhibition of mitochondrial respiration may represent the first step in the induction of antidepressant side effects associated with disturbed metabolism and transport of these drugs.

According to the neurotrophic hypothesis of depression, the therapeutic action of long-term treatment by antidepressants and mood stabilizers is associated with the induction of enhanced neuroplasticity and a final neurotrophic effects. All these processes require a great amount of cellular energy. Thus, drug-induced changes of mitochondrial functions could play a significant role in their mechanisms of action. We hypothesized that the increase of mitochondrial respiration is coupled with the therapeutic effects of antidepressants and the decrease of respiratory rate could be related to adverse effects of pharmacotherapy. On the basis of observations that mitochondrial dysfunction induces activation of gene expression through the transcription factor, cAMP response element-binding protein (CREB) via the cAMP signaling pathway (Arnould et al., 2002) and that effects of long-term treatment with antidepressants are linked to the activation of the “CAMP/protein kinase A/CREB/brain-derived neurotrophic factor” pathway (D'Sa
it can be speculated that antidepressant-induced mitochondrial dysfunction could be involved in early biochemical processes leading to changes in neuroplasticity. The hypothesis that the weak antimitochondrial actions of antidepressants could provide a potentially protective pre-conditioning effect (Calabrese et al., 2010; Abdel-Razaq et al., 2011), in which antidepressant–induced mitochondrial dysfunction below the threshold of injury results in subsequent protection should be tested.

Effects of antidepressants and mood stabilizers are comprised of marked changes in mitochondrial functions. Previously, we found that monoamine oxidase, citrate synthase and complexes I and IV were the most affected and are suggested as candidates in the search for new biological markers of mood disorders, targets of new antidepressants or predictors of response to pharmacotherapy (Fišar et al., 2010; Hroudova and Fisar, 2010). In the present study, respiratory rate of intact mitochondria was found to be an appropriate parameter for the sensitive measurement of drug effects on cellular bioenergetics. It has been assumed that the decrease in mitochondrial respiratory rate may be implicated in some of the effects of these drugs on neuroplasticity and/or in their adverse effects. The exact physiological role of respiratory rate inhibition by tested antidepressants is unknown. It can be speculated that inhibition of respiratory rate by pharmacologically different antidepressants could contribute to the regulation of mood and emotions or to the interindividual differences in drug response. Although it is always difficult to extrapolate from in vitro models to clinical reality, the present results suggest that the inhibitory effect of antidepressants on mitochondrial respiratory rate cannot be ignored.

5. Conclusions

Evidently, based on our results, the effect of antidepressants (amitriptyline, fluoxetine, tianeptine) on mitochondrial respiratory rate was inhibitory, whereas the effect of mood stabilizers (lithium, valproate, olanzapine) was negligible. Moreover, there were significant differences between several tested drugs, regardless of the similar physicochemical properties of their molecules. This indicates the existence of a certain selectivity of antidepressant–mitochondria interactions.

In conclusion, antidepressants, but not mood stabilizers, are potent partial inhibitors of mitochondrial respiration energized using substrates of ETC complex I or complex II. Under the same experimental conditions, antidepressants exert similar inhibitory potency as chlorpromazine. The presented results indicate that

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**Fig. 2.** Drug-induced inhibition of respiratory rate in pig brain mitochondria incubated with substrates for electron supply through complex II. Changes in respiratory rate induced by antidepressants (A) and mood stabilizers (B) were measured. Dose–response curves are displayed as plots of the respiratory rate against the drug concentration. The samples were continuously stirred and incubated at 37°C; titration with drugs was achieved in 3 min intervals. Following subtraction of residual oxygen consumption from respiratory rates at different drug/medium concentrations, differences among drug-titrated and medium-titrated samples were calculated and relative drug-induced changes in respiratory rate were determined, presuming that the relative respiratory rate equals to 1 at zero drug concentration. Half maximal inhibitory concentration (IC50) and Hill slope were calculated using nonlinear regression analysis software (Table 2). Values are means from at least 4 independent measurements ± standard deviation. Lines represent the best fitted curves using the four-parameter logistic function.
lithium, valproate, olanzapine, ketamine and propranolol can be considered as safe compounds in relation to drug-induced changes in the mitochondrial respiratory rate and to related changes in the energy metabolism of brain mitochondria. Antidepressants can be considered as less safe compounds in this sense. However, a systematic in vivo investigation of antidepressant or mood stabilizing effect on the respiratory rate is necessary to confirm this clearly important conclusion. The effect of newly synthesized psychotropic drugs on mitochondrial respiration should be included in their testing in order to uncover their mitochondrial toxicity or potential neurotoxic effects. High-resolution respirometry is a suitable sensitive technique for these measurements.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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