Biochemical Pharmacology 78 (2009) 85–95

Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

Mitochondrial complex I as a novel target for intraneuronal DA: Modulation of respiration in intact cells

Hanit Brenner-Lavie, Ehud Klein, Dorit Ben-Shachar *

Research Laboratory of Psychobiology, Department of Psychiatry, Rambam Medical Center, Bruce Rappaport Faculty of Medicine, Technion IIT, Haifa, Israel

ARTICLE INFO

Article history:
Received 1 February 2009
Accepted 24 March 2009

Keywords:
Human neuroblastoma SH-SY5Y cell
respiration
Mitochondria
Complex I
Dopamine
Antipsychotic drugs
ROS

ABSTRACT

Accumulating evidence suggests a role for mitochondria in synaptic potentiation and neurotransmission as well as in morphogenesis and plasticity of spines and synapses. However, studies investigating the ability of neurotransmitters to reciprocally affect mitochondrial function are sparse. In the present study we investigated whether dopamine can affect mitochondrial function in intact neuronal cells. We have shown that short- or long-term exposure of human neuroblastoma SH-SY5Y cells to dopamine (DA) inhibited mitochondrial respiration. This inhibition was associated with an increase in DA intracellular levels, and was prevented by the DA membrane transporter inhibitors, cocaine and GBR-12909. DA inhibited respiration driven through complex I but not through complexes II or III, in line with DA ability to specifically inhibit complex I activity in mitochondrial preparations. The effect of DA on complex I was not associated with altered expression of three subunits of complex I, which were formerly reported abnormal in DA-related pathologies. DA effects on respiration were not due to its ability to form reactive oxygen species. Antipsychotic drugs, which compete with DA on its receptors and inhibit complex I activity, also decreased complex I driven mitochondrial respiration. These findings may suggest that DA, which is taken up by neurons, can affect mitochondria and thereby neurotransmission and synaptic plasticity. Such a mechanism may be of relevance to DA-related non-degenerative pathologies such as schizophrenia.

1. Introduction

Neuronal cells are highly dependent on aerobic oxidative phosphorylation and ATP production in order to maintain their function. Mitochondria are the energy source that drives the biochemical processes involved in various cell functions. In addition to ATP production, mitochondria regulate intracellular Ca^{2+} concentration [1], which has been shown to play an important role in the generation of action potentials and calcium signaling processes, including synaptic transmission, cytoskeletal dynamics, and activity dependent regulation of gene expression. Previous studies utilizing pharmacological tools to inhibit mitochondria revealed defects in synaptic potentiation and a failure to maintain neurotransmission under rigorous stimulation [2,3]. Further evidence for the critical role of mitochondria in neuronal activity is the finding that loss of mitochondria from axon terminals result in defective synaptic transmission in *Drosophila* [4–6] and that dendritic mitochondria are essential in the morphogenesis and plasticity of spines and synapses in hippocampal tissue slices [7]. Studies on the ability of neurotransmitters to reciprocally affect mitochondrial function and ATP production process in a cellular system have focused mainly on the effect of glutamate on mitochondrial function [8–10]. Two *in vivo* studies suggest that DA may also interfere with the mitochondrial respiratory system, demonstrating that elevated rat brain DA concentrations following chronic administration of L-DOPA or D-methamphetamine resulted in a reduction in striatal ATP [11,12]. In addition, it has been shown that DA can dissipate mitochondrial membrane potential in B lymphocytes and in human neuroblastoma cells [13,14]. In isolated mitochondria derived from rat brain and muscle, DA, its precursor L-DOPA or its metabolite DOPAC, were shown to interfere with mitochondrial respiration. This has been attributed to their ability to be metabolized to highly reactive oxygen species (ROS) that can cause a state of oxidative stress [15–18]. However, in disrupted mitochondria from both rat brain and human platelets, it was shown that DA *per se* reversibly inhibited complex I activity but not that of complex II, IV and V [13,19,20]. These findings suggest that DA can also interfere with mitochondrial function by a direct interaction with complex I. In line with the latter are our recent results demonstrating that intact mitochondria can accumulate DA, thereby enabling the interaction between DA and complex I [13].

* Corresponding author at: Laboratory of Psychobiology, Department of Psychiatry, Rambam Medical Center and B. Rappaport Faculty of Medicine, Technion ITT, P.O.B 9649 Haifa, 31096, Israel. Tel.: +972 4 8295204; fax: +972 4 8295220.
E-mail address: shachar@tx.technion.ac.il (D. Ben-Shachar).

0006-2952/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2009.03.024

Interestingly, antipsychotic drugs, which compete with DA on its receptors, inhibit complex I activity both *in vivo* and *in vitro*. Thus, administration of antipsychotic drugs to brain slices of humans or mice as well as to isolated rat brain mitochondria, reduced complex I activity [21–24]. In addition, chronic antipsychotic treatment of rats or mice caused a reduction in complex I activity in different brain regions [22,23,25,26]. It is notable that in schizophrenia, in which antipsychotic drugs are the common practice, abnormalities have been reported in DA transmission and in complex I activity as well as in its subunits encoded by nuclear DNA genes [25,27–36]. We hypothesize that an interaction between DA and complex I, which affects mitochondrial activity without the induction of an oxidative stress state, may modulate neuronal functioning. The results of the present study show that short- and long-term increases in intracellular DA decreased oxygen consumption via its interaction with complex I in intact SH-SY5Y cells. DA-induced inhibition of respiration was not associated with the formation of ROS, or with altered expression of three subunits of complex I. Antipsychotic drugs showed similar inhibitory effects on respiration. These findings suggest that DA–complex I interaction is functionally manifested in a cellular system, and may be a relevant mechanism in DA related non-degenerative pathologies such as schizophrenia, in which DA and mitochondria as well as abnormal neuronal plastic processes are considered as part of the pathophysiology.

2. Materials and methods

2.1. Materials

All drugs were purchased from Sigma Chemical Company, St. Louis, MO, USA. MitoSOX and H_2DCFDA were purchased from Molecular Probes (Eugene, USA). Media for tissue culture was purchased from Biological Industries (Beit Haemek Ltd., Israel). All other materials were of the highest purity and were purchased from standard commercial sources.

2.2. Cell treatments

Human neuroblastoma SH-SY5Y cells (10^6 cell) were plated in Modified Eagle's Medium (MEM, with non-essential amino-acids)/F12(HAM)(1:1) supplemented with 10% fetal calf serum, 2% sodium bicarbonate, 1% sodium pyruvate and 1% penicillin/streptomycin at 37 °C in a 5% CO_2 humidified incubator. Prior to drug treatment cells were preincubated with or without 0.1 mM tranylcypromine (MAO A and B inhibitor) for 30 min and then the medium was replaced as will be specified for each experiment. For long lasting effects of drugs cells were treated with either a single or repeated (every 24 h) administration of DA (10^{-7} to 10^{-5} M), haloperidol (10^{-7} to 10^{-5} M) or rotenone (1 nM) for 6 h, 24 h, 48 h or 72 h. Control cells received an equivalent volume of solvent (DMSO or PBS) for the same length of time.

Cell viability was determined in the presence of 0.1% Tryptan Blue, and counted in a haemocytometer.

2.3. Respiration

Oxygen utilization was measured polarographically with a thermostatically controlled (37 °C) Clark oxygen electrode (Strathkelvin 782 Oxygen System; Strathkelvin Instrument Ltd., Scotland). Cells ($2–5 \times 10^6$) were washed and suspended in a serum-free DMEM low glucose medium without phenol red (standard medium) or in Hank's balanced salt solution (HBSS) and were added to a 1 ml water-jacketed chamber. Cell respiration was determined in the presence of: rotenone (5 μM); the uncoupler CCCP (5 μM); DA (10^{-4} to 10^{-8} M) or antipsychotic drugs (5×10^{-5} to 10^{-4} M: haloperidol, clozapine, chlorpromazine) for up to 40 min. Measurements were taken online and all control incubations were performed in an identical manner. Respiration rates are expressed as micromole O_2 per liter per hour. Oxygen consumption through each enzymatic complex of the respiratory chain was measured separately in 1 ml of standard medium (pH 7.4) containing 0.3 M mannitol, 10 mM KCl, 5 mM $MgCl_2$, 10 mM KH_2PO_4 and 0.25 mg bovine serum albumin. Cell membrane was permeabilized with digitonin (0.001%) in order to avoid the uptake mechanism for each of the substrates as previously described [37]. Respiration rates were assessed after the addition of the following substrates and inhibitors: pruvate/glutamate (5 mM; complex I substrate) plus malate (1 mM; complex II inhibitor) for complex I induced respiration. Succinate (20 mM; complex II substrate) in the presence of rotenone (5 μM) and ATP (0.4 mM) for complex II induced respiration, and glycerol-3-phosphate (10 mM; complex III substrate) in the presence of rotenone (4 μM), ATP (0.4 mM) and malonate (20 mM) for complex III induced respiration.

2.4. Intracellular DA

Intracellular DA was measured by HPLC with an electrochemical detector (ESA Inc., MA, USA). Cells were incubated as specified for the respiration reaction in the presence or absence of three different concentrations of DA (5×10^{-5} to 5×10^{-4}) and of 5 mM succinate. The reaction was terminated by centrifugation at $280 \times g$ at room temperature for 15 min. The pellet was washed three times with excess of buffer and centrifuged between each wash to avoid contamination by extracellular DA. The final pellet was placed on ice and dissolved in 100 μl of 0.1 M $HClO_4$. After freezing and thawing the homogenate was centrifuged at $12{,}000 \times g$ at 4 °C, and aliquots of the supernatant were analyzed with HPLC. The electrodetection was performed at +0.3 V. The mobile phase contained 0.1 M NaH_2PO_4, 0.27 mM Na_2EDTA, 1 mM octanesulfuric acid, 4.5% acetonitrile, 2.5% methanol (pH 2.75). Data were analyzed using Borwin software [38].

2.5. Reactive oxygen species (ROS) production

The formation of intracellular ROS was measured by incubating SH-SY5Y cells (2×10^5cells) suspended in HBSS in the presence of 5-(and-6)-carboxy-2',7'-dichlorodihydrofluorescein diacetate (H_2DCFDAfinal concentration 25 μM) for 30 min at 37 °C [39]. H_2DCFDA is a stable non-fluorescent molecule that passively diffuses into cells, where the acetate can be cleaved from the parent molecule by intracellular esterases to produce a polar diol that is well retained within the cells. The diol can then be oxidized by ROS to a fluorescent form. Redox state of cells was determined by exciting H_2DCFDA-loaded cells at 485 nm and detection of emitted fluorescence at 520 nm using FLUOstar OPTIMA (BMG Labtech, Germany). After loading, DA (10^{-6} or 10^{-4} M) was added to the cells and measurements were obtained every 5 min for up to 60 min. H_2O_2 (10^{-4} M) was used as a positive control.

Superoxide formation was measured by incubating SH-SY5Y cells (2×10^5cells) suspended in HBSS using red mitochondrial superoxide indicator (MitoSOX final concentration 5 μM) for 10 min at 37 °C [40]. Superoxides were determined by exciting MitoSOX-loaded cells at 485 nm and detection of emitted fluorescence at 580 nm using FLUOstar OPTIMA. After loading, DA (10^{-6} or 10^{-4} M) was added to the cells and measurements were obtained every 5 min for up to 60 min. Rotenone (10 μM) was used as a positive control.

2.6. RT-PCR analysis

RNA extraction from cells was performed using RNA STAT-60 kit (TEL-TEST, Inc., Frienwood, TX, USA) followed by additional

Table 1
Primer sequences and PCR conditions.

mRNA		Primer sequence	Denaturing temperature and time °C (s)	Annealing temperature and time °C (s)	Elongation temperature and time °C (s)	Number of cycles	Product size (bp)
NDUFV2	S NS	5′-GGAGGAGCTTTATTTGTGCAC-3' 5′-CCTGCTTGTACACCAAATCC-3′	94 (60)	55 (60)	72 (60)	35	640
NDFUV1	S NS	5′-TACATCCGAGGGGAATTCTACA-3′ 5′-GTTCTTTCAAGGGCACAGACAT-3′	94 (60)	60 (60)	72 (60)	35	426
NDUFS1	S NS	5′-TACTCGCTGCATCAGGTTTG-3′ 5′-CATGCATACGTGGCAAAATC-3′	94 (60)	58 (60)	72 (60)	35	299
β-Actin	S NS	5′-TGAAGTGTGACGTGGACATCCG-3′ 5′-GCTGTCACCTTCACCGTTCCAG-3′	94 (60)	60 (60)	72 (60)	25	447
18S-RNA	S NS	5′-AGGAATTGACGGAAGGGCAC-3′ 5′-GTGCAGCCC CGGACATCTAAG-3′	94 (60)	60 (60)	72 (60)	25	324

All templates were initially denatured for 5 min at 94 °C, and after completing all cycles, were extended a final extension of 10 min at 72 °C.

purification using phenol:chloroform:isoamyl alcohol (25:24:1) and isopropranol. All RNA preparations were dissolved in RNase free water and were then treated by DNase as described previously [32,41]. The final RNA preparation was dissolved in RNase free water and stored in −80 °C until use. RNA integrity depicted in the form of three bands corresponding to 28S, 18S and 5S RNA was assessed by electrophoresis in a 1% agarose/formaldehyde gel stained with ethidium bromide. The amount and purity of extracted RNA was determined spectophotometricaly. The expression of *NDUFV1*, *NDUFV2* and *NDUFS1* encoding for the 51 kDa, 24 kDa and the 75 kDa subunits of complex I, respectively, was studied by using RT-PCR [31,32]. Amplification of RT-cDNA was first performed on a control specimen at different concentrations to define a linear range for all genes. The number of cycles, cDNA amount and primers concentration was established according to a stringent calibration process determining the log-linear phase of amplification for each gene. After establishing the optimal reaction conditions, PCR amplification was performed. PCR products were stained with ethidium bromide separated by electrophoresis on 2% agarose gel and analyzed by densitometer. Sequences of PCR primers for *NDUFV2*, *NDUFV1*, *NDUFS1* and β-actin were designed according to sequence obtained from Gene-Bank (Table 1). β-Actin was used for normalizing variations in RNA aliquots taken for RT reactions. A single batch of RNA isolated from untreated cells, on which PCR was performed at three different concentrations was assayed in parallel with each set of samples as a positive control and as a control for the log-linear phase of amplification for each reaction.

2.7. Immunolotting

Protein was extracted from cells as previously described [31]. Briefly, cells were homogenized in 10 mM Tris buffer pH 7.4 containing 250 mM sucrose, 5 mM EDTA, 0.5% NP40 and TM protease inhibitors cocktail. Following centrifugation (16,000 × *g*, 10 min) the supernatant (80 or 100 μg total protein was diluted 1:1 in electrophoresis sample buffer 250 mM Tris–HCl, pH 6.8 containing 20% (v/v) glycerol, 4% (w/v) sodium dodecyl sulfate, 10% (v/v) 2-mercaptoethanol and 0.5 mg/ml bromophenol blue. The protein sample was separated on SDS polyacrylamide gel and transferred to a nitrocellulose membrane. Quality of protein transfer was assayed by Ponceau staining. Following blocking (T-TBS containing 2% Bovine serum albumin) of nonspecific binding sites, membranes were incubated at 4 °C overnight with primary recombinant rabbit antihuman antibody Protein samples were separated on SDS-PAGE, transferred to a nitrocellulose membrane and quality of transfer was assayed by Ponceau staining. Primary recombinant rabbit antihuman antibodies used; anti-24 kDa 1:1500, anti-51 kDa 1:500, anti-75 kDa 1:1000 synthesized by Sigma–Aldrich, Israel. Secondary antibodies used; anti rabbit-IgG 1:15,000 for 24 kDa, 1:10,000 for 51 kDa and 75 kDa subunits (Santa Cruz Biotechnology, Santa Cruz, CA). β-Actin was used for normalizing variations in protein aliquots. Blots were developed with Amersham's ECL, exposed to XLS Kodak film and analyzed by densitometer. β-Actin was used for normalizing variations in protein aliquots. In addition, a single batch of rat brain mitochondrial protein in three different concentrations was used as a positive control and for normalization. Protein concentration was measured using Bradford reagent (BIO-RAD, Munchen, Germany).

2.8. Data analysis

Statistical significance was determined by one-way ANOVA followed by Bonferroni post hoc multiple comparisons test. SPSS 14.0 program was used for all analyses.

3. Results

3.1. DA inhibits respiration of SH-SY5Y cells

Optimal conditions for the measurement of respiration of SH-SY5Y neuroblastoma cells were first established. Fig. 1A depicts a representative respiration graph of control respiration and the effects of complex I inhibitor, rotenone (1 μM), which inhibited respiration by approximately 50% (50.2 ± 2.4%, $p < 0.001$), and of the uncoupler CCCP (5 μM), which enhanced respiration by 169.9 ± 16.9% ($p < 0.001$) (Fig. 1A). DA inhibited respiration in a dose-dependent manner, at a concentration range that was previously shown to have no effect on SH-SY5Y cell viability [20] (Fig. 1B). To study the specificity of DA induced effect on respiration, two additional monoamines, 5-HT and norepinephrine (NE), were analyzed. DA (10^{-4} M) significantly inhibited respiration by 35.1 ± 9.5% ($p < 0.001$), NE (10^{-4} M) was significantly less effective and inhibited cells respiration by 8.62.7% ($p < 0.001$ vs. control and $p < 0.01$ vs. DA) while 5-HT (10^{-4} M) did not cause any significant inhibition of respiration (Fig. 1C). These experiments were done in the presence of the MAO inhibitor tranylcyoromine.

In the absence of tranylcyoromine, DA (10^{-4} M) was less effective in decreasing SH-SY5Y cells oxygen consumption (20 ± 1.4% $p < 0.0001$ vs. control and $p < 0.00$ vs. DA without TC). The DA uptake inhibitor, cocaine (10 mM) almost completely prevented DA inhibitory effect on respiration (5.2 ± 0.6%, $p = 0.001$ vs. control) (Fig. 2A and B). To further study the role of DA membrane transporter (DAT) in DA-induced inhibition of respiration, LLC PK1 cells (derived from pig kidney) which lack DAT were compared to those stably transfected with human DAT (h-DAT). Our results show

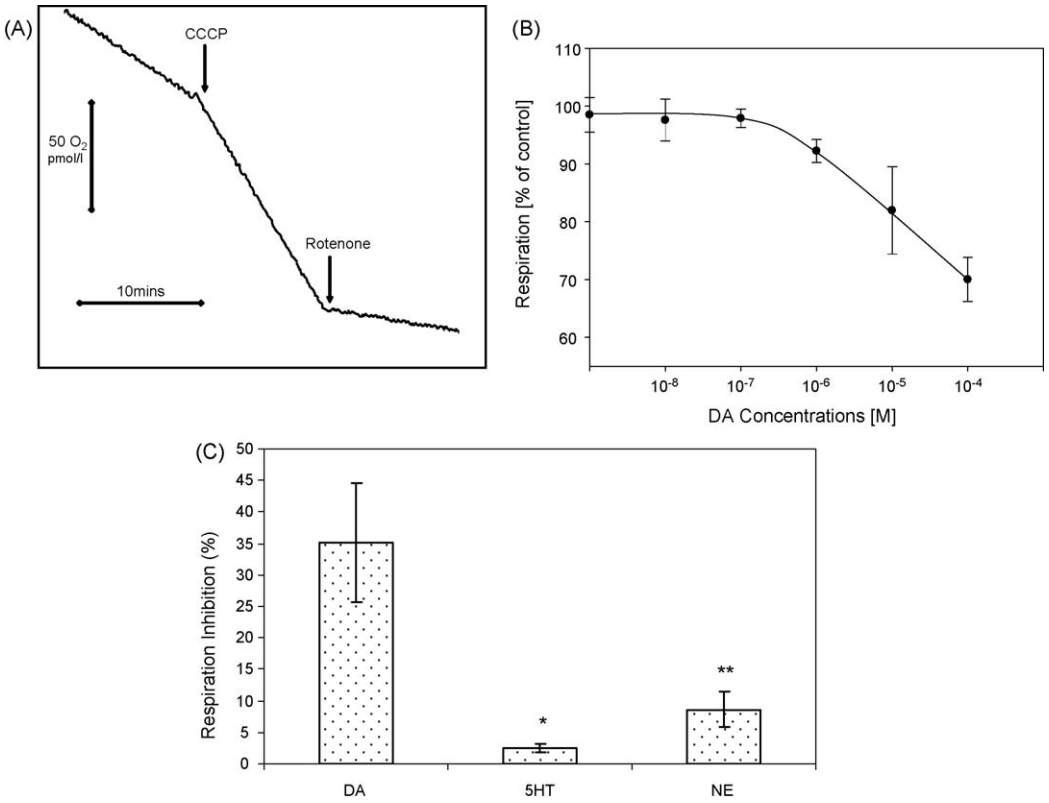

Fig. 1. Mitochondrial respiration in the presence or absence of DA, 5-HT and NE. (A) A representative graph of cell respiration and the effect of 5 μM CCCP and 1 μM rotenone. (B) DA induced dose-dependent inhibition of respiration. (C) 5-HT and NE effects on cells respiration. Respiration was measured during 10 min of incubation polarographically using a thermostatically controlled (37 °C) Clark oxygen electrode in SH-SY5Y cells. A concentration of 10^{-4} M was used for all monoamines. Results are means ± SD of 4 experiments. The significant difference between treatments was analyzed by one-way ANOVA $F(4,2) = 23.386$, $p < 0.0006$, followed by Bonferroni post hoc test. $^{*}p = 0.01$; $^{**}p = 0.015$ vs. DA.

that DA significantly inhibits respiration of the h-DAT transfected cells by 14.6 ± 6.1% ($p = 0.0001$), but not that of LLC PK1 cell (Fig. 2C).

3.2. Intracellular DA concentration

To study whether intracellular concentrations of DA are increased under our assay conditions and are at a physiological relevant range, DA was analyzed by HPLC. Basal levels of DA, in cells suspended in 1 ml serum-free medium and treated with 0.1 mM tranylcypromine, was 0.143 ± 0.05 pmol/10^6 cells. Following incubation with 10^{-4} M DA for 10 min, intracellular DA levels increased to 17.1 ± 3.8 pmol/10^6 cells ($p < 0.001$), therefore less than 0.1% of the extracellular DA was taken-up by 10^6 cells. Although tranylcypromine was used to inhibit MAO, a small amount of DOPAC was still formed in the presence of 10^{-4} M DA and was about 14% of DOPAC formed in the absence of the inhibitor (2.05 ± 0.34 pmol/10^6 cells vs. 14.64 ± 1.8 pmol/10^6 cells). In the absence of tranylcypromine intracellular DA concentrations were 10-fold less than that in the presence of the MAO inhibitor, while a 5-fold increase was observed in DOPAC concentrations. In the presence of cocaine (10^{-4} M), or both GBR-12909 (10^{-5} M) and desipramine (10^{-5} M) (DAT and NET inhibitors respectively), intracellular concentrations of DA significantly decreased to 6.33 ± 0.99 pmol/10^6 cells ($^{*}p < 0.029$) and to 2.12 ± 0.53 pmol/10^6 cells ($^{**}p < 0.019$), respectively as compared to DA treated cells (Fig. 2D).

3.3. Long-term effects of DA on respiration

To verify whether DA effects are long lasting, SH-SY5Y neuroblastoma cells were exposed to a single or repeated (every 24 h) administration of DA. DA (10^{-5} M) in the presence of tranylcypromine significantly decreased oxygen consumption following 24 h and 48 h of incubation by 41 ± 0.1% ($p < 0.001$) and 42 ± 0.2% ($p < 0.007$), respectively. Cocaine almost completely prevented DA induced inhibition of respiration, as after 24 h, respiration was not significantly different from the control untreated cells, while significantly different from DA treated cells (13 ± 0.02% $p < 0.034$ vs. DA). After 48 h, cocaine's ability to prevent DA induced inhibition was attenuated as respiration, although not reaching a significance difference from the control cells, was inhibited by 31 ± 0.2%. Moreover, haloperidol (10^{-5} M), which is an antipsychotic drug with high affinity to DA D2 receptor and is an inhibitor of complex I activity, decreased oxygen consumption in viable SH-SY5Y neuroblastoma cells after 24 h and 48 h of incubation by 32 ± 0.1% ($p < 0.013$) and 34 ± 0.03%, respectively (Fig. 3). Both DA and haloperidol did not affect cell viability under our various assay conditions.

3.4. ROS formation in the presence of DA

To determine whether ROS formation is involved in the effects of DA on respiration, we analyzed superoxide and H_2O_2 production in SH-SY5Y cells treated with DA with or without tranycypromine, either following short- (up to 60 min) or long-term exposure to DA (up to 48 h). During the 60 min time interval no significant increase in superoxide production was observed in the presence of DA. On the contrary, a small but still significant reduction in superoxide formation (15%, $p < 0.0019$ vs. control) was observed in cells treated with 10^{-4} M DA, with no change in the presence of 10^{-6} M DA (Fig. 4A). Following 24 and 48 h of incubation with DA, in the presence or absence of the MAO inhibitor, tranylcypromine,

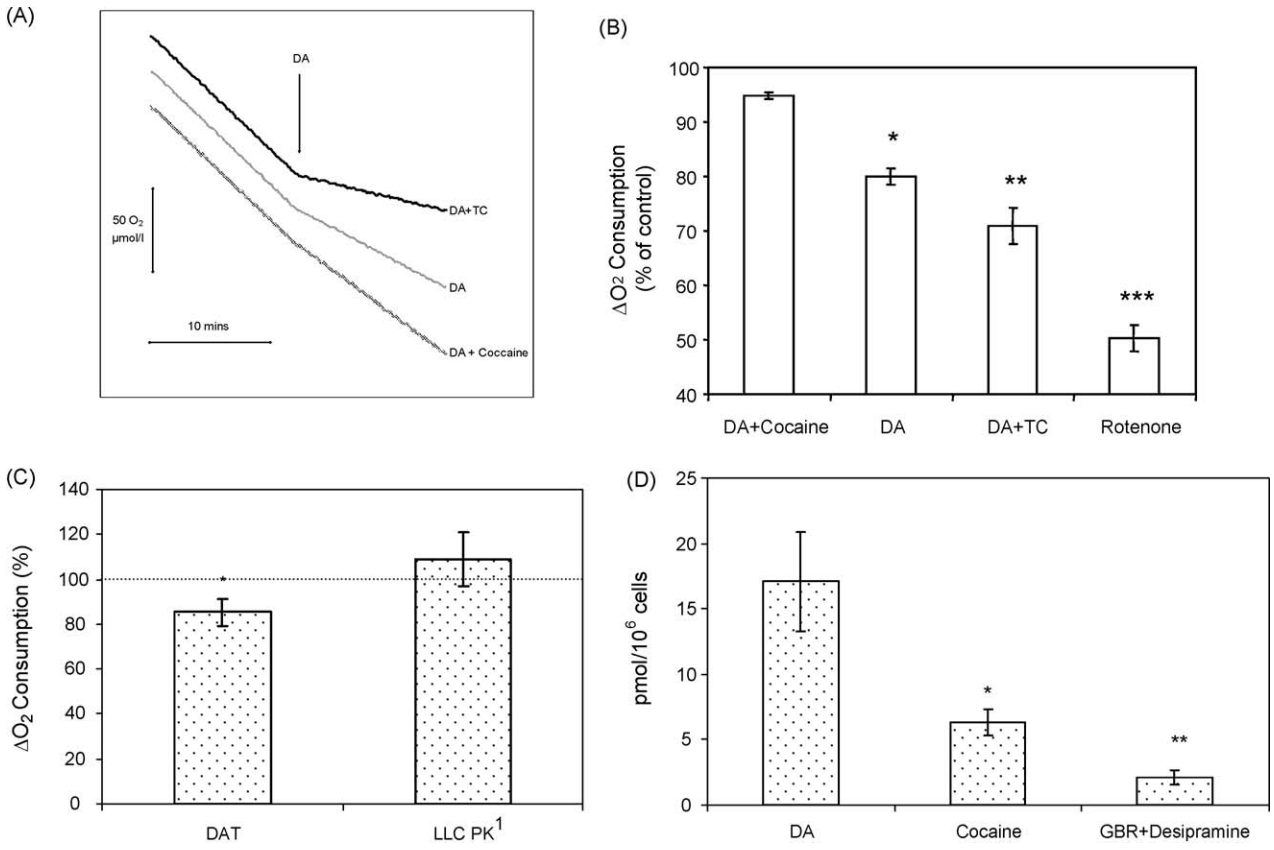

Fig. 2. Intracellular DA inhibits cellular respiration. (A) A representative graph of SH-SY5Y respiration in the presence of 10^{-4} M DA, with or without 10^{-4} M tranylcypromine (TC) or 10^{-4} M cocaine. (B) Quantification of the effect of DA on respiration presented in (A). Results are means ± SD of 8–10 different experiments. The significant difference between treatments was analyzed by one-way ANOVA $F(4,34) = 42.385$, $p < 0.0001$ followed by Bonferroni post hoc test. $^{*}p < 0.002$; $^{**}p < 0.002$; $^{***}p < 0.001$ vs. control. $p < 0.001$ DA vs. DA + TC; DA vs. rotenone; DA + TC vs. rotenone. (C) DA (10^{-4} M) effect on respiration in LLC PK1 cells and LLC PK1 cells transfected with human DAT. Results are means ± SD of 3 experiments. The significant difference between groups was analyzed by Student's *t*-test. $^{*}p = 0.005$. (D) DA intracellular concentrations in cells measured by HPLC. DA intracellular concentrations were measured in the presence of 10^{-4} M TC+ 10^{-4} M DA, 10^{-4} M cocaine, 10^{-5} M GBR-12909+ desipramine. Results are means ± SD of 4 experiments. The significant difference between groups was analyzed by one-way ANOVA $F(2,11) = 7.344$, $p < 0.009$ followed by Bonferroni post hoc test. $^{*}p < 0.029$, $^{**}p < 0.019$ vs. DA.

superoxides were not formed. Haloperidol significantly increased superoxide formation only after 24 h ($p < 0.0001$ vs. DA and vs. control + tranylcypromine) (Fig. 4B).

H_2O_2 production was not increased by DA incubation for up to 60 min. On the contrary DA prevented the increase in H_2O_2 formation following 60 min of incubation ($p < 0.0001$ vs. control). Incubation with DA or with haloperidol for 24 h significantly increased H_2O_2 production (148% and 140% respectively of control; $p < 0.0001$). Addition of tranylcypromine prevented DA induced H_2O_2 production. In the presence of 10^{-5} M cocaine and tranylcypromine DA caused a significant increase in H_2O_2 production (174% of control + tranylcypromine $p < 0.0001$) following 24 h of incubation, similar to the effect of DA alone at this time point. This may suggest that extracellular DA is responsible for the production of H_2O_2. Interestingly, following 48 h incubation with DA (with or without tranylcypromine), a significant decrease in H_2O_2 production was observed (55% and 61% of control, respectively, $p < 0.0001$), suggesting that after 48 h, cellular protective mechanisms have already been activated (Fig. 4C and D). In line with the latter is the lack of effect of haloperidol following 48 h.

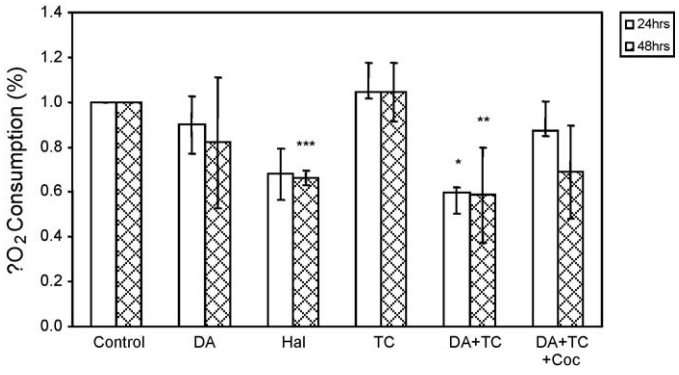

Fig. 3. DA long-term effects on respiration in SH-SY5Y cells. Respiration rates were assessed after 24–48 h of incubation with 10^{-5} M DA with and without tranylcyromine, cocaine, or haloperidol. Data are means ± SD of 4 experiments in duplicates. The significant difference between treatments was analyzed by one-way ANOVA $F(5,12) = 14.382$, $p < 0.000$ for 24 h, $F(5,10) = 10.065$, $p < 0.001$ for 48 h, followed by Bonferroni post hoc test, for 24 h $^{*}p < 0.002$ vs. control; $^{*}p < 0.034$ vs. Tc + DA + Coc; $^{*}p < 0.001$ vs. Tc; for 48 h $^{**}p < 0.013$ vs. control; $^{***}p < 0.012$ vs. control.

3.5. DA effect on respiration driven through complexes I, II or III

In order to study whether DA effect on respiration in intact cells can be linked to its ability to inhibit complex I activity as previously observed in disrupted mitochondria [20], cellular oxygen consumption rates were measured while pushing respiration through each enzymatic complex of the respiratory chain separately (Fig. 5A). Respiration driven through complex I was inhibited by DA by 33.3 ± 0.07% ($p < 0.0006$) but not that driven through complex II or through complex III (Fig. 5B). Similarly, both typical and atypical antipsychotic drugs specifically inhibited complex I driven mitochondrial respiration (Table 2), but had no effect when respiration was driven through complex II.

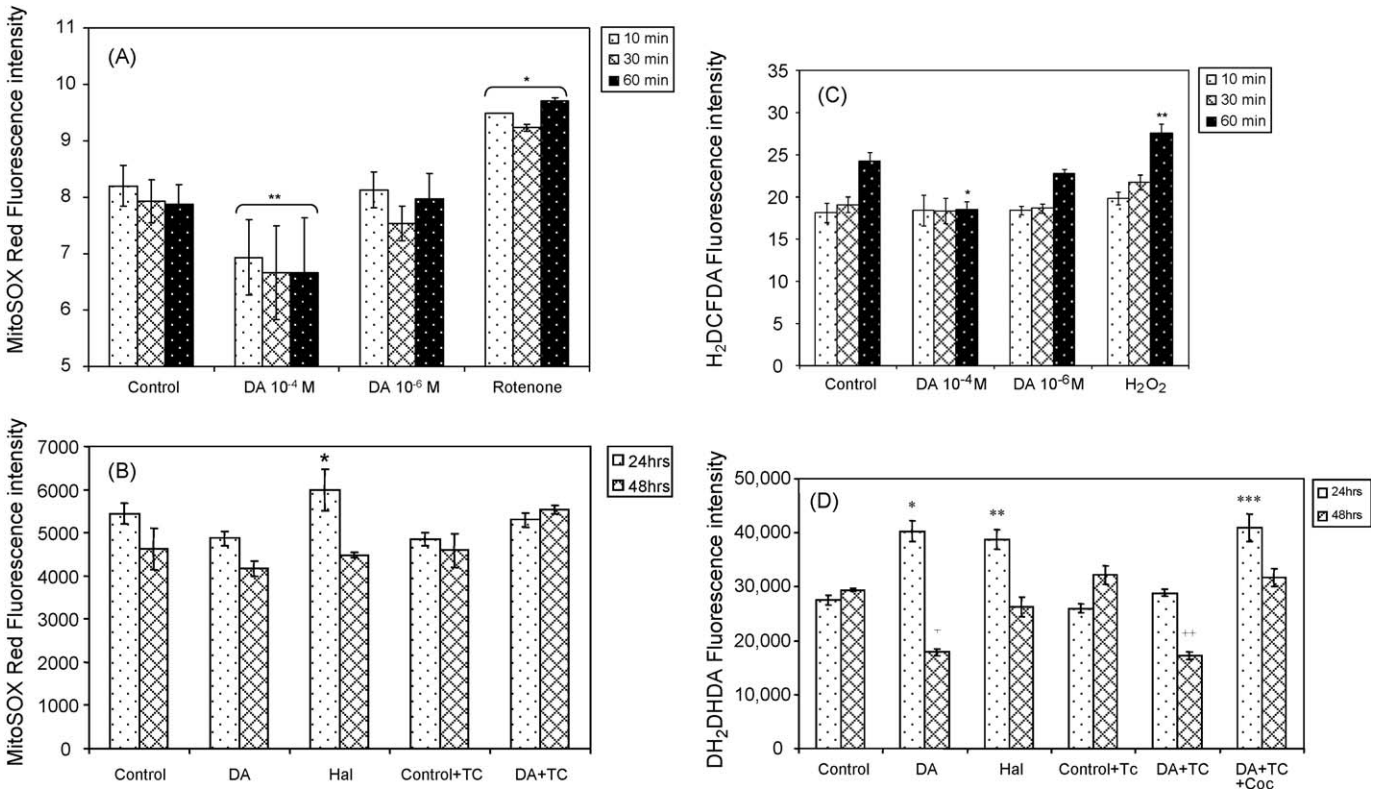

Fig. 4. Time dependent DA induced ROS formation in SH-SY5Y cells. DA did not induce the formation of superoxides. H_2O_2 was formed only after 24 h under conditions that did not affect cellular respiration. (A) Superoxide formation for up to 60 min of DA exposure. Results are means ± SD of 4–6 experiments. The significance of the difference was analyzed by one-way ANOVA $F(3,8) = 19.5$; 14.66;14.69 (for 10 min, 30 min and 60 min respectively) $p < 0.0001$ followed by Bonferroni post hoc test $^{*}p < 0.028$; $^{**}p < 0.019$ vs. control. (B) Superoxide formation after log-term exposure to DA and haloperidol (Hal). Results are means ± SEM of 4–6 experiments in triplicates. The significance of the difference was analyzed by one-way ANOVA $F(5,18) = 26.506$, $p < 0.0001$ for 24 h, $F(5,18) = 2.86$, $p < 0.045$ for 48 h, Bonferroni post hoc test, 24 h, $^{*}p < 0.0001$ Hal vs. DA and control + Tc. For superoxide detection SH-SY5Y cells were loaded with MitoSOX. Cells were incubated with DA with or without TC, haloperidol or rotenone as described in Section 2. (C) H_2O_2 formation for up to 60 min of DA exposure. Results are means ± SD of 4–6 experiments. The significance of the difference was analyzed by one-way ANOVA $F(3,8) = 48.95$ (for 60 min), $p < 0.0001$ followed by Bonferroni post hoc test; $^{*}p = 0.0001$, $^{**}p = 0.015$ vs. control. (D) H_2O_2 formation after log-term exposure to DA and haloperidol. Results are means ± SEM of 5 experiments in triplicates. The significance of the difference was analyzed by one-way ANOVA $F(5,18) = 131.36$, $p < 0.0001$ for 24 h, $F(5,18) = 107.86$, $p < 0.0001$ for 48 h, followed by Bonferroni post hoc test; for 24 h $^{*}p = 0.0001$ vs. control; $^{**}p = 0.0001$ vs. control; $^{***}p = 0.0001$ vs. DA + TC; for 48 h $^{+}p = 0.0001$ vs. control; $^{++}p = 0.0001$ vs. control + TC. For H_2O_2 formation SH-SY5Y cells were loaded with H_2DCFDA. Cells were incubated with DA with or without TC or haloperidol as described in Section 2.

3.6. Effects of DA on mRNA and protein expression of complex I subunits

Our previous studies have shown that the mRNA and protein expression of complex I subunits were altered in schizophrenia both in periphery and in post mortem brain specimen [29,32]. In order to study whether inhibition of cellular respiration following long-term exposure of cells to DA or haloperidol is associated with impaired expression of complex I subunits, SH-SY5Y cells were treated with 10^{-7} to 10^{-5} M DA or haloperidol for 6, 24 and 48 h. Our results suggest that both mRNA and protein levels of all three subunits were unaffected by DA (Fig. 6A) or by haloperidol (Fig. 6B). In line with the latter, a single administration of non-toxic doses of rotenone (0.1 nM), which was shown to inhibit respiration, had no effect on mRNA levels of complex I subunits in human neuroblastoma SH-SY5Y cells up to 48 h after treatment (Fig. 6C). Repeated administration of DA for up to 72 h also had no effect on mRNA levels of the three subunits of complex I (Fig. 6D).

Table 2
Antipsychotic drugs affect respiration through complex I inhibition.

Drug	% Inhibition—complex I		% Inhibition—complex II	
	5×10^{-5} M	10^{-4} M	5×10^{-5} M	10^{-4} M
Haloperidol	27.2 ± 4.7*	47.6 ± 10.0*	0.7 ± 0.7	2.9 ± 1.8
Clozapine	31.2 ± 5.6*	39.9 ± 20.8*	10.9 ± 1.6	2.6 ± 2.4
Chlorpromezine	20.9 ± 18.1*	43.8 ± 17.3*	6.6 ± 3.5	14.9 ± 2.1

The significance of inhibition of respiration through complex I by haloperidol, clozapine and chlorpromazine was calculated by student *t*-test. Values are means ± SD of 4–6 detections.
* $p < 0.00$.

4. Discussion

The present study provides novel evidence for the ability of intracellular DA to inhibit mitochondrial function through its interaction with complex I in intact viable human neuroblastoma SH-SY5Y cells, which was not associated with the production of ROS. DA significantly decreased cellular oxygen consumption already 10–15 min after treatment, in line with previous findings using FACS and confocal microscopy, demonstrating a significant dissipation of $\Delta\psi_m$ by DA, with no effect on cell survival [13,14]. DA inhibitory effects were intensified by the MAO inhibitor, tranylcypromine, which increases free intracellular DA concentrations. In line with the latter, DA-induced impairment of respiration was prevented in the presence of cocaine, which inhibits DA uptake both by DA and NE plasma membrane transporters (DAT and NET, respectively). The role of DAT in DA induced alterations of respiration was further strengthened by the findings that DA significantly inhibited respiration of the h-DAT transfected LLC PK1 cells, but had no effect on the common parental LLC PK1 cells, which lack active biogenic amines transporters [42,43]. Taken together, these results suggest that the intracellular DA, which is increased following synaptic release and reuptake, is involved in mitochondrial dysfunction.

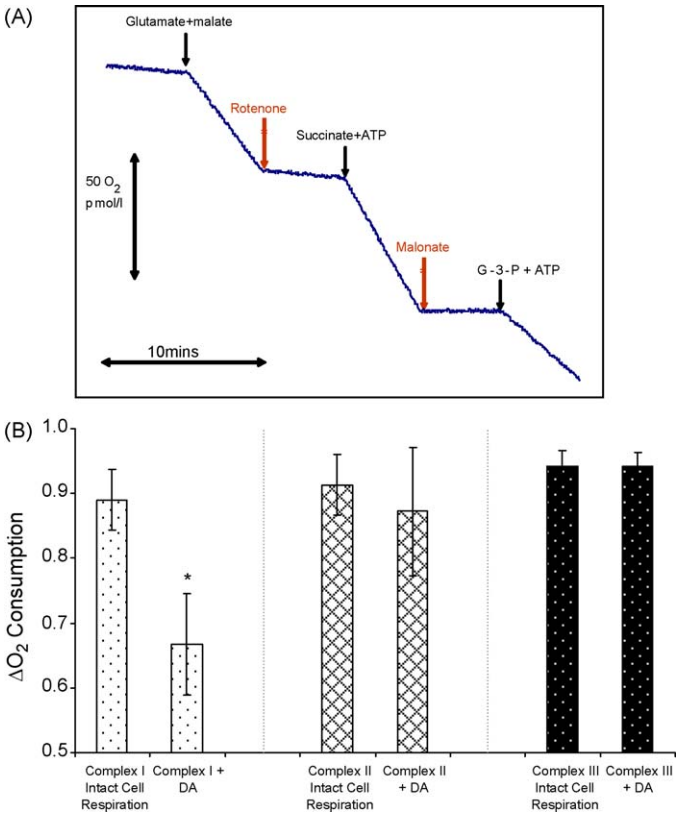

Fig. 5. Oxygen consumption through each of the enzymatic complexes of the respiratory chain in the presence or absence of DA. (A) A representative graph of the respiration rates for each enzyme of the respiratory chain. Respiration was assessed after the permeabilization of cellular membrane with 0.001% digitonin and the addition of the appropriate substrates and inhibitors as described in Section 2. (B) Quantification of DA effects after 10 min of incubation on respiration through each of the complexes. Results are means ± SD of 5 experiments. The significant differences were analyzed by *t*-test $^{*}p < 0.0006$.

Unlike DA, other monoamines had a marginal effect on cellular respiration. NE showed a small inhibitory effect, which is in line with NE ability to be taken up by intact isolated mitochondria [13] and to inhibit complex I activity, although less efficiently than DA [20]. The limited effect of NE corroborate with the findings that NE had no effect on intracellular ATP levels in SH-SY5Y cells, as opposed to DA, which caused a dose-dependent decrease in ATP production [20]. Serotonin had no significant effect on cell respiration, which is probably due to its inability to be taken up by intact isolated mitochondria as well as to inhibit mitochondrial complex I activity [13,20].

Concomitant with the importance of free intracellular DA for the induction of mitochondrial dysfunction was the significant increase in intracellular levels of DA measured by HPLC, following cell exposure to DA. In addition, the prevention of DA inhibitory effect on respiration by cocaine or by GBR-12909 + desipramine (specific DAT and NET, respectively) was associated with a significant decrease in intracellular DA concentrations. Although tranylcypromine was used to inhibit DA metabolism by MAO, a small amount of DOPAC was still formed. This finding cannot exclude the involvement of DOPAC in the inhibition of respiration. Previous reports showed that DOPAC can inhibit respiration in isolated mitochondria [17,44], as well as complex I activity in disrupted mitochondria, though with lower IC_{50} than DA [20]. However, under our assay conditions, DOPAC levels were about 10% of the amount of intracellular DA, implying that the effect of DOPAC on respiration was minor.

In the present study, cells were treated with 10^{-4} M DA. This concentration is higher than that reported by microdialysis studies in which extracellular DA concentrations can range between 3 and 750 nM depending on neuronal activation and brain region [45,46]. Yet it is lower than synaptic concentrations of DA, which can range between 30 and 100 mM following activation of DA cell bodies [47]. In addition, in previous studies using SH-SY5Y cells as well as isolated mitochondria, the concentration generally used for DA was at the range of 5×10^{-5} to 10^{-4} M. At such concentrations, exposure of cells to DA for 12–18 h did not affect cell viability or caused apoptosis [20,48,49]. Finally, under pathological conditions which are associated with substantial release of DA, as in schizophrenia [50], mitochondria enriched nerve terminals are exposed to high DA levels due to its increased re-uptake and synthesis [51].

Numerous studies have shown ctyotoxic effects following exposure of various cell types to DA for 24 h or longer [52–57]. Therefore, the long-term effect of DA on mitochondrial respiration was studied in cells exposed to repeated DA administration in the presence of tranylcypromine for 24–48 h. Similar to its immediate effects, DA significantly decreased oxygen consumption in SH-SY5Y neuroblastoma cells, which was prevented by cocaine and was depended on the presence of tranylcypromine. The lack of effect of DA in the absence of tranylcypromine contradict previous reports in isolated mitochondria, which suggested that inhibition of MAO prevents the ability of DA to inhibit mitochondrial respiration [16,17,49]. This apparent contradiction may be attributed to the fact that isolated mitochondria react differently to DA or its metabolites from mitochondria in their natural surrounding milieu. Likewise, the MAO inhibitor, depranyl, was previously shown to protect rats from death following intraventricullar injection of DA [19].

Many studies attributed DA deleterious effects on mitochondrial function to its catabolism by MAO to H_2O_2, or due to its auto-oxidation to reactive quinones [58,59]. In this study however, exposure of cells to DA was not associated with H_2O_2 formation. These results are not totally unexpected as cells were pretreated with the MAO A and B inhibitor, tranylcypromine, significantly reducing the formation of DOPAC and therefore also the formation of H_2O_2. HPLC results show that under our assay conditions 0.5 μM DOPAC was formed in cells exposed to DA, indicating that intracellular levels of H_2O_2 were elevated by 0.5 μM at most. It was previously reported that physiological concentrations of H_2O_2 are in the range of 25–60 μM and that 100 μM H_2O_2 for at least 1.5 h are needed to induce alterations in $\Delta\psi_m$ [60]. Following 24 h of DA incubation, however, H_2O_2 production was significantly increased in the absence of tranylcypromine and was normalized following 48 h of DA treatment. As respiration was not reduced in the absence of tranylcypromine, these results further support that H_2O_2 is not involved in the inhibition of respiration in intact cells and is probably formed extracellularly as cocaine did not prevent H_2O_2 formation. The generation of reactive quinones cannot be totally excluded. However, DA-induced inhibition of cell respiration was already observed after several minutes, while previous findings demonstrated that more than 2 h were needed for a sufficient build-up of the damaging quinones to produce inactivation of OXPHOS in cells [61]. In addition, it was reported that DA quinones increase state 4 respiration, which is indicative of proton leakage across the inner membrane of the mitochondria (uncoupling) [49]. If this was the case, we would expect an increase in oxygen consumption in the presence of DA, which contradicts our observations.

It is well established that inhibition of the respiratory chain can also induce the production of superoxides. However, under our various experimental conditions we could not detect superoxide formation following both brief and long-term exposure of cells to DA whether with or without tranylcpromine. This suggests that either superoxides were not generated in association with DA

inhibition of respiration, or that the superoxides were detoxified by mitochondrial or cellular protective mechanisms. Taken together, the results hitherto point to a ROS-independent mechanism by which DA interferes with mitochondrial respiration in cells, which is intensified by the inhibition of MAO.

The present data show that in intact cells, respiration driven through complex I but not through complex II or complex III was inhibited by DA. Further support for the involvement of complex I in DA induced inhibition of respiration, is DA ability to specifically inhibit complex I activity in disrupted mitochondria, possibly by interacting at a site located between the binding site for NADH and the iron-sulfur cluster N1 [20], but not the activity of complexes II, IV and V [13,20]. Interestingly, we have recently demonstrated that DA can cross the highly selective mitochondrial inner membrane and be accumulated by synaptosomal-free, intact, coupled and respiring mitochondria, enabling its interaction with complex I [13]. Although other sites of interaction between DA and mitochondria, prior to complex I, cannot be ruled-out based on our previous findings and the present results, it is conceivable that DA is able to inhibit complex I, a major determinant of mitochondrial respiration [62], and can thereby lead to mitochondrial dysfunction.

Experimental evidence raises the possibility that DA can also affect complex I at the level of its subunit expression. For example, in schizophrenia DA abnormal transmission as well as impairments in mitochondrial genes, including those encoding for complex I, were reported [31–33,63,64]. Therefore, we tested whether the long-term effects of DA on respiration are associated with alterations in three genes encoding for the 24-, 51-, and 75-kDa subunits of complex I, located at the suggested site of its

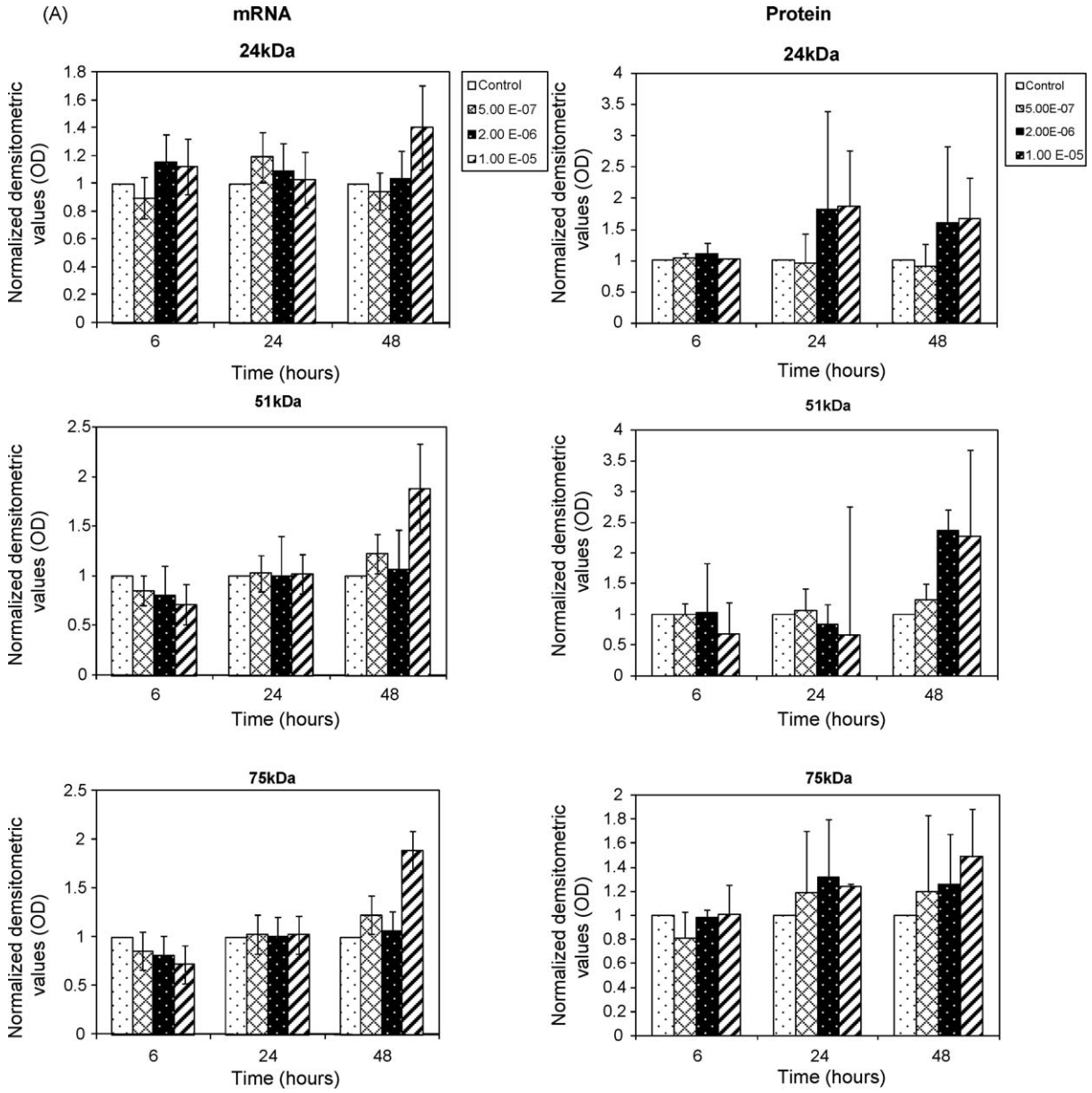

Fig. 6. DA and haloperidol effect on mRNA and protein levels of complex I subunits in human neuroblastoma SH-SY5Y cells. Time dependent effect of a single administration of (A) DA or (B) Haloperidol (Hal). 5×10^{-7} to 10^{-5} M DA or Hal was applied and mRNA and protein levels were analyzed by RT-PCR and immunoblotting, respectively after 6, 24 and 48 h. DA as well as haloperidol showed no significant effect on mRNA or protein levels of complex I subunits. Data are means ± SD of 4 experiments in triplicates. (C) Rotenone effect on mRNA levels of complex I subunits. No significant effect on mRNA levels of complex I subunits was observed following a single administration of rotenone (0.1 nM) after 24 and 48 h. Data are means ± SD of 3 experiments in triplicates. (D) The effect of repeated administration of DA on mRNA of complex I subunits. No significant effect on mRNA levels of complex I subunits was observed following daily administration of 10^{-5} M DA for up to 72 h Data are means ± SD of 3–4 experiments in triplicates.

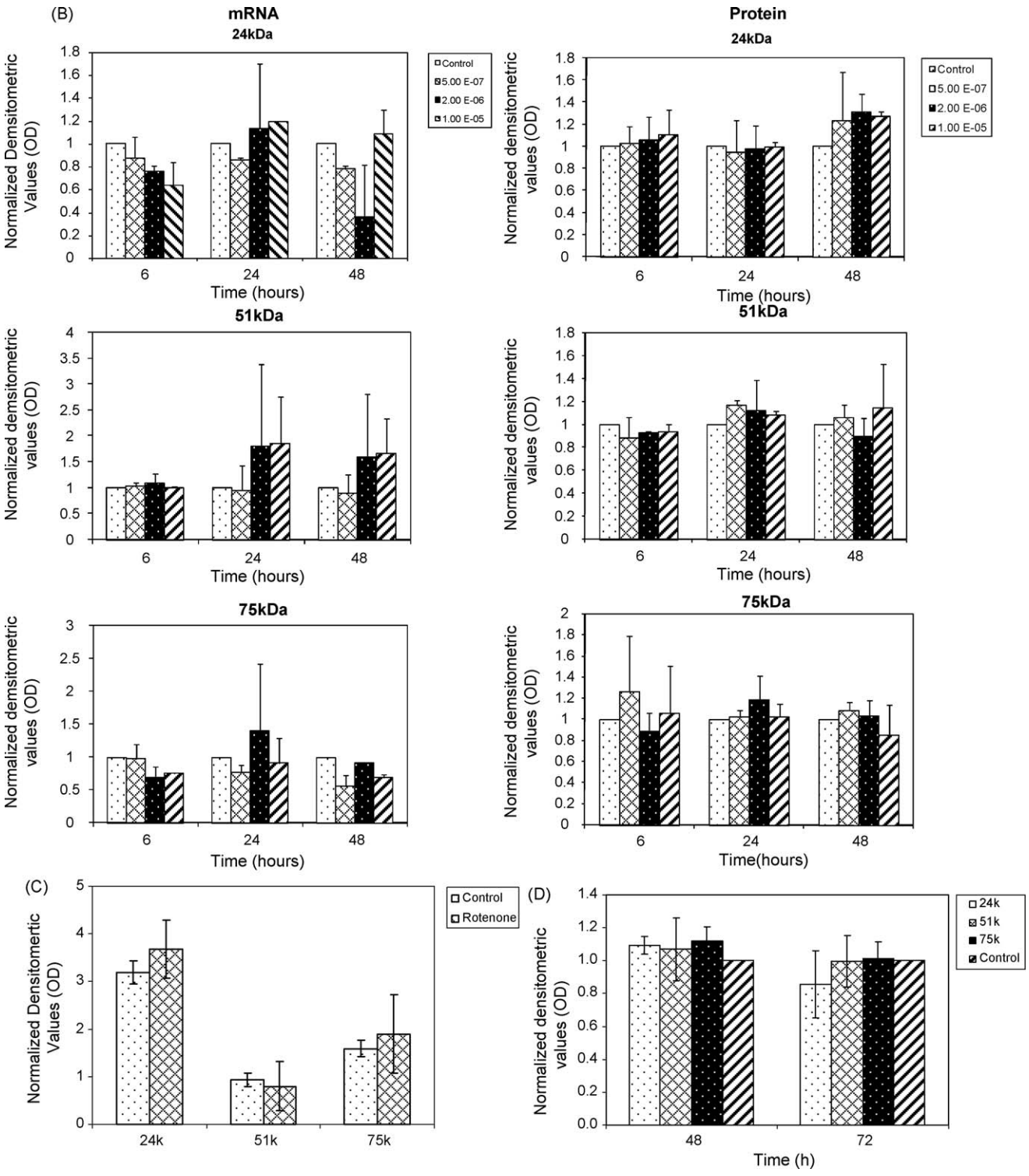

Fig. 6. (*Continued*).

interaction with DA. No change was observed in both mRNA and protein levels of all three subunits of complex I, up to 72 h following a single or repeated administration of DA. Similarly, rotenone did not affect mRNA levels of these subunits. However, to ultimately exclude DA effects on complex I gene expression, longer exposure to DA may be necessary. Antipsychotic drugs, which are DA D2 receptor antagonists, have also been shown to directly inhibit complex I activity both in experimental models and in human tissue [21–24,65]. Similar to DA and rotenone, exposing cell for up to 48 h to the typical potent antipsychotic drug haloperidol had no effect on mRNA and protein levels of the three subunits of complex I. However, both haloperidol and clozapine, typical and atypical antipsychotic drugs, respectively, specifically inhibited complex I driven mitochondrial respiration, but not that through complex II. These findings may explain the decrease observed in complex I driven respiration in peripheral blood mononuclears isolated from schizophrenic patients chronically treated with haloperidol [66,67]. Interestingly, two main mechanisms have

been suggested for haloperidol-induced effect. One involves a direct inhibition of complex I by haloperidol pyridinum metabolite (HPP+) with structural similarities to the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [68,69]. The other mechanism involves the oxidation of protein essential thiol groups to disulfides by haloperidol [65,67]. We therefore suggest that similarly to haloperidol, DA can also interfere with mitochondrial respiration in a dual manner, via the formation of ROS or by a direct interaction with complex I. The former was suggested as a possible mechanism for the neurodegenerative processes associated with Parkinson's disease. However, via the latter mechanism DA can modulate mitochondria dependent intracellular Ca^{2+} buffering and ATP formation, which are highly important for neuronal activity. Neuronal activity can monitor synaptic connectivity associated with adaptive changes in emotional and cognitive function. It is therefore tempting to hypothesize that abnormal DA–complex I interaction may be a part of the pathological processes observed in non-degenerative disorders such as schizophrenia.

Acknowledgments

This work was supported by a grant from the Chief Scientist of the Israeli Ministry of Health. We thank Dr. G. Rudnick (Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut) for the LLC-PK1 cells.

References

[1] Werth JL, Thayer SA. Mitochondria buffer physiological calcium loads in cultured rat dorsal root ganglion neurons. J Neurosci 1994;14:348–56.
[2] Alnaes E, Rahamimoff R. On the role of mitochondria in transmitter release from motor nerve terminals. J Physiol 1975;248:285–306.
[3] Tang Y, Zucker RS. Mitochondrial involvement in post-tetanic potentiation of synaptic transmission. Neuron 1997;18:483–91.
[4] Stowers RS, Megeath LJ, Gorska-Andrzejak J, Meinertzhagen IA, Schwarz TL. Axonal transport of mitochondria to synapses depends on milton, a novel Drosophila protein. Neuron 2002;36:1063–77.
[5] Verstreken P, Ly CV, Venken KJ, Koh TW, Zhou Y, Bellen HJ. Synaptic mitochondria are critical for mobilization of reserve pool vesicles at Drosophila neuromuscular junctions. Neuron 2005;47:365–78.
[6] Guo X, Macleod GT, Wellington A, Hu F, Panchumarthi S, Schoenfield M, et al. The GTPase dMiro is required for axonal transport of mitochondria to Drosophila synapses. Neuron 2005;47:379–93.
[7] Li Z, Okamoto K-I, Hayashi Y, Sheng M. The importance of dendritic mitochondria in the morphogenesis and plasticity of spines and synapses. Cell 2004;119:873–87.
[8] Greenwood SM, Mizielinska SM, Frenguelli BG, Harvey J, Connolly CN. Mitochondrial dysfunction and dendritic beading during neuronal toxicity. J Biol Chem 2007;282:26235–44.
[9] White RJ, Reynolds IJ. Mitochondrial depolarization in glutamate-stimulated neurons: an early signal specific to excitotoxin exposure. J Neurosci 1996;16:5688–97.
[10] Garcia O, Almeida A, Massieu L, Bolanos JP. Increased mitochondrial respiration maintains the mitochondrial membrane potential and promotes survival of cerebellar neurons in an endogenous model of glutamate receptor activation. J Neurochem 2005;92:183–90.
[11] Przedborski S, Jackson-Lewis V, Muthane U, Jiang H, Ferreria M, Naini AB, et al. Chronic levodopa administration alters cerebral mitochondrial respiratory chain activity. Ann Neurol 1993;34:715–23.
[12] Chan P, Di Monte DA, Luo JJ, DeLanney LE, Irwin I, Langston JW. Rapid ATP loss caused by methamphetamine in the mouse striatum: relationship between energy impairment and dopaminergic neurotoxicity. J Neurochem 1994;62:2484–7.
[13] Brenner-Lavie H, Klein E, Zuk R, Gazawi H, Ljubuncic P, Ben-Shachar D. Dopamine modulates mitochondrial function in viable SH-SY5Y cells possibly via its interaction with complex I: relevance to dopamine pathology in schizophrenia. Biochim Biophys Acta 2008;1777:173–85.
[14] Elkashef AM, Al-Barazi H, Venable D, Baker I, Hill J, Apud J, et al. Dopamine effect on the mitochondria potential in B lymphocytes of schizophrenic patients and normal controls. Prog Neuropsychopharmacol Biol Psychiatry 2002;26:145–8.
[15] Berman SB, Hatings TG. Dopamine oxidation alters mitochondrial respiration and induces transition in brain mitochondria: implications for Parkinson's disease. J Neurochem 1999;73:1127–37.
[16] Cohen G, Farooqui R, Kesler N. Parkinson's Disease: a new link between monoamine oxidase and mitochondrial electron flow. Proc Natl Acad Sci USA 1997;94:4890–4.
[17] Gluck MR, Zeevalk GD. Inhibition of brain mitochondrial respiration by dopamine and its metabolites: implications for Parkinson's disease and catecholamine-associated diseases. J Neurochem 2004;91:788–95.
[18] Porta F, Takala J, Weikert C, Kaufmann P, Krahenbuhl S, Jakob SM. Effect of endotoxin, dobutamine and dopamine on muscle mitochondrial respiration in vitro. J Endotoxin Res 2006;12:358–66.
[19] Ben-Shachar D, Zuk R, Glinka Y. Dopamine neurotoxicity: inhibition of mitochondrial respiration. J Neurochem 1995;64:718–23.
[20] Ben-Shachar D, Zuk R, Gazawi H, Ljubuncic P. Dopamine toxicity involves mitochondrial complex I inhibition: implications to dopamine-related neuropsychiatric disorders. Biochem Pharmacol 2004;67:1965–74.
[21] Burkhardt C, Kelly JP, Lim YH, Filley CM, Parker WD. Neuroleptic medications inhibit complex I of the electron transport chain. Ann Neurol 1993;33:512–7.
[22] Prince JA, Yassin MS, Oreland L. Neuroleptic-induced mitochondrial enzyme alterations in the rat brain. J Pharmacol Exp Ther 1997;280:261–7.
[23] Balijepalli S, Kenchappa RS, Boyd MR, Ravindranath V. Protein thiol oxidation by haloperidol results in inhibition of mitochondrial complex I in brain regions: comparison with atypical antipsychotics. Neurochem Int 2001;38:425–35.
[24] Maurer I, Moller HJ. Inhibition of complex I by neuroleptics in normal human brain cortex paralles the extrapyramidal toxicity of neuroleptics. Mol Cell Biochem 1997;174:255–9.
[25] Whatley SA, Curi D, Das Gupta F. Superoxide, neuroleptics and the ubiquinone and cytochrome b5 reductases in brain and lymphocytes from normal and schizophrenic patients. Mol Psychiatry 1998;3:227–37.
[26] Barrientos A, Marin C, Miro O, Casademont J, Gomez M, Nunes V, et al. Biochemical and molecular effects of chronic haloperidol administration on brain and muscle mitochondria of rats. J Neurosci Res 1998;53:475–81.
[27] Ben-Shachar D. Mitochondrial dysfunction in schizophrenia: a possible linkage to dopamine. J Neurochem 2002;83:1241–51.
[28] Ben-Shachar D, Laifenfeld D. Mitochondria, synaptic plasticity, and schizophrenia. Int Rev Neurobiol 2004;59:273–96.
[29] Ben-Shachar D, Zuk R, Gazawi H, Reshef A, Sheinkman A, Klein E. Increased mitochondrial complex I activity in platelets of schizophrenic patients. Int J Neuropsychopharmacol 1999;2:245–53.
[30] Maurer I, Zierz S, Moller H. Evidence for a mitochondrial oxidative phosphorylation defect in brains from patients with schizophrenia. Schizophr Res 2001;48:125–36.
[31] Karry R, Klein E, Ben Shachar D. Mitochondrial complex I subunits expression is altered in schizophrenia: a postmortem study. Biol Psychiatry 2004;55:676–84.
[32] Dror N, Klein E, Karry R, Sheinkman A, Kirsh Z, Mazor M, et al. State dependent alterations in mitochondrial complex I activity in platelets: a potential peripheral marker for schizophrenia. Mol Psychiatry 2002;7:995–1001.
[33] Mulcrone J, Whatley SA, Ferrier IN, Marchbanks RM. A study of altered gene expression in frontal cortex from schizophrenic patients using differential screening. Schizophr Res 1995;14:203–13.
[34] Cavelier L, Jazin E, Eriksson I, Prince J, Bave B, Oreland L, et al. Decreased cytochrome c oxidase activity and lack of age related accumulation of mtDNA in brain of schizophrenics. Genomics 1995;29:217–28.
[35] Mehler-Wex C, Duvigneau JC, Hartl RT, Ben-Shachar D, Warnke A, Gerlach M. Increased mRNA levels of the mitochondrial complex I 75-kDa subunit: a potential peripheral marker of early onset schizophrenia? Eur Child Adolesc Psychiatry 2006.
[36] Washizuka S, Kakiuchi C, Mori K, Kunugi H, Tajima O, Akiyama T, et al. Association of mitochondrial complex I subunit gene NDUFV2 at 18p11 with bipolar disorder. Am J Med Genet 2003;120:72–8.
[37] Hofhaus G, Shakeley RM, Attardi G. Use of polarography to detect respiration defects in cell cultures. Methods Enzymol 1996;264:476–83.
[38] Ben-Shachar D, Klein E, Tabak A, Finberg JPM. Effect of single and repeated ministration of fluvoxamine on noradrenaline release in rat brain. Eur J Pharmacol 1997;332:237–42.
[39] Yu Z, Li W, Hillman J, Brunk UT. Human neuroblastoma (SH-SY5Y) cells are highly sensitive to the lysosomotropic aldehyde 3-aminopropanal. Brain Res 2004;1016:163–9.
[40] Abramov AY, Scorziello A, Duchen MR. Three distinct mechanisms generate oxygen free radicals in neurons and contribute to cell death during anoxia and reoxygenation 10.1523/JNEUROSCI.4468-06.2007. J Neurosci 2007;27:1129–38.
[41] Laifenfeld D, Karry R, Klein E, Ben-Shachar D. Alterations in cell adhesion molecule L1 and functionally related genes in major depression: a postmortem study. Biol Psychiatry 2005;57:716–25.
[42] Gu H, Wall SC, Rudnick G. Stable expression of biogenic amine transporters reveals differences in inhibitor sensitivity, kinetics, and ion dependence. J Biol Chem 1994;269:7124–30.
[43] Gorentla BK, Vaughan RA. Differential effects of dopamine and psychoactive drugs on dopamine transporter phosphorylation and regulation. Neuropharmacology 2005;49:759–68.
[44] Nunes C, Almeida L, Laranjinha J. Synergistic inhibition of respiration in brain mitochondria by nitric oxide and dihydroxyphenylacetic acid (DOPAC): implications for Parkinson's disease. Neurochem Int 2005;47:173–82.
[45] Kalivas PW, Duffy P. Time course of extracellular dopamine and behavioral sensitization to cocaine. I. Dopamine axon terminals. J Neurosci 1993;13:266–75.
[46] Zetterstrom T, Sharp T, Marsden CA, Ungerstedt U. In vivo measurement of dopamine and its metabolites by intracerebral dialysis: changes after D-amphetamine. J Neurochem 1983;41:1769–73.

[47] Lavin A, Nogueira L, Lapish CC, Wightman RM, Phillips PE, Seamans JK. Mesocortical dopamine neurons operate in distinct temporal domains using multimodal signaling. J Neurosci 2005;25:5013–23.
[48] Gimenez-Xavier P, Gomez-Santos C, Castano E, Francisco R, Boada J, Unzeta M, et al. The decrease of NAD(P)H has a prominent role in dopamine toxicity. Biochim Biophys Acta 2006;1762:564–74.
[49] Berman SB, Hastings TG. Dopamine oxidation alters mitochondrial respiration and induces permeability transition in brain mitochondria: implications for Parkinson's disease. J Neurochem 1999;73:1127–37.
[50] Laruelle M, Abi-Dargham A, Gil R, Kegeles L, Innis R. Increased dopamine transmission in schizophrenia: relationship to illness phase. Biol Psychiatry 1999;46:56–72.
[51] Lindstrom LH, Gefvert O, Hagberg G, Lundberg T, Bergstrom M, Hartvig P, et al. Increased dopamine synthesis rate in medial prefrontal cortex and striatum in schizophrenia indicated by L-(beta-11C) DOPA and PET. Biol Psychiatry 1999;46:681–8.
[52] Offen D, Ziv I, Panet H, Wasserman L, Stein R, Melamed E, et al. Dopamine-induced apoptosis is inhibited in PC12 cells expressing bcl-2. Cell Mol Neurobiol 1997;17:289–304.
[53] Tanaka M, Sotomatsu A, Kanai H, Hirai S. DOPA and dopamine cause cultured neuronal death in the presence of iron. J Neurochem 1991;101:198–203.
[54] Cohen G. Oxidative stress in the nervous system. In: Sies H, editor. Oxidative stress. London: Academic Press; 1985. p. 383–401.
[55] McLaughlin BA, Nelson D, Erecinska M, Chesselet MF. Toxicity of dopamine to striatal neurons in vitro and potentiation of cell death by a mitochondrial inhibitor. J Neurochem 1998;70:2406–15.
[56] Rosenberg PA. Catecholamine toxicity in cerebral cortex of dissociated cell culture. J Neurosci 1988;8:2887–94.
[57] Weinreb O, Mandel S, Youdim MBH. cDNA gene expression profile homology of antioxidants and their antiapoptotic and proapoptotic activities in human neuroblastoma cells. FASEB J 2003;17:935–7.
[58] LaVoie MJ, Hastings TG. Dopamine quinone formation and protein modification associated with the striatal neurotoxicity of methamphetamine: evidence against a role for extracellular dopamine. J Neurosci 1999;19: 1484–91.
[59] Izumi Y, Sawada H, Yamamoto N, Kume T, Katsuki H, Shimohama S, et al. Iron accelerates the conversion of dopamine-oxidized intermediates into melanin and provides protection in SH-SY5Y cells. J Neurosci Res 2005;82:126–37.
[60] Moor AN, Gottipati S, Mallet RT, Sun J, Giblin FJ, Roque R, et al. A putative mitochondrial mechanism for antioxidative cytoprotection by 17beta-estradiol. Exp Eye Res 2004;78:933–44.
[61] Khan FH, Sen T, Maiti AK, Jana S, Chatterjee U, Chakrabarti S. Inhibition of rat brain mitochondrial electron transport chain activity by dopamine oxidation products during extended in vitro incubation: implications for Parkinson's disease. Biochim Biophys Acta 2005;1741:65–74.
[62] Davey GP, Peuchen S, Clark JB. Energy thresholds in brain mitochondria: potential involvement in neurodegeneration. J Biol Chem 1998;273:12753–7.
[63] Middleton FA, Mirnics K, Pierri JN, Lewis DA, Levitt P. Gene expression profiling reveals alterations of specific metabolic pathways in schizophrenia. J Neurosci 2002;22:2718–29.
[64] Prabakaran S, Swatton JE, Ryan MM, Huffaker SJ, Huang JT, Griffin JL, et al. Mitochondrial dysfunction in schizophrenia: evidence for compromised brain metabolism and oxidative stress. Mol Psychiatry 2004;9:684–97. 43.
[65] Balijepalli S, Boyd MR, Ravindranath V. Inhibition of mitochondrial complex I by haloperidol: the role of thiol oxidation. Neuropsychopharmacology 1999;38:567–77.
[66] Casademont J, Garrabou G, Miro O, Lopez S, Pons A, Bernardo M, et al. Neuroleptic treatment effect on mitochondrial electron transport chain: peripheral blood mononuclear cells analysis in psychotic patients. J Clin Psychopharmacol 2007;27:284–8.
[67] Burkhardt Barrientos A, Marin C, Miro O, Casademont J, Gomez M, Nunes V, et al. Biochemical and molecular effects of chronic haloperidol administration on brain and muscle mitochondria of rats. J Neurosci Res 1998;53:475–81.
[68] Subramanyam B, Woolf F, Castagnoli N. Studies on the in vivo conversion of haloperidol to a potentially neurotoxic pyridinium metabolite. Chem Res Toxicol 1991;4:123–8.
[69] Rollema H, Skolnik M, D'engelbronner J, Igarashi K, Usuki E, Castagnoli N. MPP+-like neurotoxicity of a pyridinium metabolite derived from haloperidol: in vivo microdialysis and in vitro mitochondrial studies. J Pharmacol Exp Ther 1994;268:380–7.