Low dose (−)deprenyl is cytoprotective: It maintains mitochondrial membrane potential and eliminates oxygen radicals

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Abstract

Hypoxia leads to a collapse in mitochondrial transmembrane potential (ΔΨM), a fall in the ATP/ADP ratio, and finally cell death. Since (−)deprenyl directly modulates ΔΨM and production of reactive oxygen species (ROS) by altering the respiratory function of mitochondria, we were interested in the dose–response relations of these effects. The changes in JC-1 red/green signal ratios (mitochondrial transmembrane potential), and the changes in the cerium staining (intracellular ROS) in hypoxic and normoxic PC12 cell cultures were measured following 1 h of Argon hypoxia and 24 h of re-oxygenation in the absence and in the presence of various concentrations of (−)deprenyl. ΔΨM shifted to lower values following hypoxia/re-oxygenation and all cells had decreased and uniform ΔΨM levels. The amount of ROS increased. Following 24 h of treatment with various concentrations of (−)deprenyl during the re-oxygenation period, survival increased, the ΔΨM shift caused by oxygen deprivation was reversed and the peroxy radical levels decreased except for at 10⁻³ M.

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Introduction

During hypoxia, the impairment of mitochondrial function, the consequent collapse in mitochondrial transmembrane potential (ΔΨM), a fall in the ATP/ADP ratio, and the hypoxia-induced cytoplasmic accumulation of cytochrome c leads to cell death (Smets et al., 1994; Kroemer et al., 1997; Yermolaieva et al., 2004; Hardie, 2003; Kim et al., 2003). The outward pumping of protons across the inner mitochondrial membrane produces a proton gradient that drives the conversion of ADP to ATP and is reflected by the ΔΨM (Sherrat, 1991). Decreased ΔΨM induces opening of the mitochondrial permeability transition pores (PTP), which may lead to the release of mitochondrial apoptosis initiation factors (AIFs) (Marchetti et al., 1996). The loss of mitochondrial membrane potential (ΔΨM) in itself may or may not lead to apoptotic cell death depending on the model system being used (Salvioli et al., 2000). An overall decrease in ΔΨM was reported to occur late in apoptosis, well after the release of cytochrome c from mitochondria (Yong et al., 1997). During re-oxygenization after a hypoxic period high amounts of reactive oxygen species (ROS) are generated (Yermolaieva et al., 2004). Mitochondria are the major generators of ROS. ROS production is associated with excessive oxidative stress. Membrane lipids are the primary targets of ROS, but proteins, carbohydrates and nucleic acids are also damaged, leading thus to cellular dysfunction and death.

(−)Deprenyl (phenyl-isopropyl-methyl-propargylamine, selegiline) is a relatively selective, irreversible inhibitor of monoamine oxidase-B (Knoll and Magyar, 1972; Birkmayer et al., 1975; Sowa et al., 2004). Deprenyl is used in the treatment of Parkinson’s disease (Oerthel and Quinn, 1997). Since (−)deprenyl directly modulates ΔΨM (Wadia et al., 1998) and thus the production of reactive oxygen species (ROS) by altering the respiratory function of mitochondria in a dose-dependent fashion (Götz et al., 1995; Thyffault et al., 1997; Wadia et al., 1998). The aim of the present study was to investigate the possible cytoprotective mechanisms of...
(−)deprenyl following hypoxia/re-oxygenization using nerve growth factor-differentiated PC12 cell culture. The present study demonstrates that (−)deprenyl improves mitochondrial function and minimizes damage in PC-12 cells subjected to hypoxia/re-oxygenation. It is most probable that this mechanism could be the basis of the protective effects of (−)deprenyl in middle cerebral artery occlusion stroke model in rat (Simon et al., 2001; Puurunen et al., 2001; Maia et al., 2004), as well as in human stroke patients (Sivenius et al., 2001).

Methods

Culturing and NGF differentiation of PC12 cells

Rat phaeochromocytoma (PC-12) cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) (Gibco BRL), supplemented with 10% (vol/vol) calf serum, 2 mM L-glutamine (Gibco BRL), penicillin (50 international units/ml), streptomycin (50 μg/ml) and PC-12 cells were predifferentiated on round cover glasses (d = 12 mm) covered with a collagen membrane prepared from acid soluble collagen isolated from rat tail (Csonka et al., 1980) for 5 days in Dulbecco’s modified Eagle’s medium (DMEM) (Gibco BRL), with 10% (vol/vol) calf serum, 5% (vol/vol) horse serum, 2 mM L-glutamine (Gibco BRL), penicillin (50 international units/ml), streptomycin (50 μg/ml) and 50 ng/ml nerve growth factor (NGF) in a humidified incubator aerated with 5% CO2 at 37 °C. All treatments of the cells were carried out in 24-well-cluster cell culture dishes with a diameter of 15 mm per well (Nunclon, Intermed, Denmark). Each well contained about 1000 cells.

Hypoxia/re-oxygenation

Cells were subjected to hypoxia and re-oxygenation as follows: hypoxia was produced by placing cultures on the bottom of an open chamber. Subsequently, it was filled with Argon gas and then the chamber was closed. A blood gas analyser (ABL Radiometer, Copenhagen) was used to control the partial O2 pressure in the cell culture medium. After 1 h of oxygen deprivation, the cultures were returned to the incubator (re-oxygenation) for 24 h. Control cultures were maintained in the incubator under normal conditions (normoxia). In the oxygen deprivation experiments, the various concentrations of (−)deprenyl to be tested were added to the culture medium right after the oxygen deprivation and remained there during the 24 h re-oxygenation period.

Assessment of cell death in PC12 cell culture with propidium iodide staining

The extent of cell death was determined by staining the cultures with 1.5 μg/ml propidium iodide dissolved in physiological saline for 2 min. The procedure was the following: the DMEM was removed and 300 μl of 1.5 μg/ml propidium iodide solution was added to the cell cultures. The numbers of viable and dead cells were counted with a fluorescence microscope using 450–490 nm excitation and 520 nm barrier filters. In order to avoid problems caused by uneven cell distribution, cellular death was expressed as a mean percentage of dead cells in three separate cultures, in twelve samples.

Combined staining procedure (JC-1 + cerium)

The changes in δψM were assessed using the lipophilic cationic membrane potential-sensitive dye JC-1, and the changes in the amounts of intracellular ROS were assessed using the cerium method. The staining procedure was the following: the DMEM was removed and 300 μl of 10 μg/ml JC-1 (Molecular Probes) solution (dissolved in physiological) saline was added to the cultures for 10 min. The staining solution was removed and the cell cultures were rinsed with physiological saline for 2 min. Then, 300 μl of 20 mmol/l CeCl3 solution (dissolved in lactated Ringer) was applied for 2 min. After the CeCl3 solution was removed the cell cultures were rinsed again with physiological saline for 2 min and subsequently the cells were fixed in 0.25% (vol/vol) glutaraldehyde solution for 2 min. The fixed cells on the round cover glasses were then covered with Vectashield mounting medium for fluorescence (Vector Laboratories, Inc. Burlingame, CA) and put on glass slides. Intracellular distribution of the dye was assessed by confocal microscopy. Fluorescence present in the cells was measured at 488-nm excitation/510- to 625-nm emission. The reaction of cerium ions with ROS forms stable, insoluble cerium perhydroxide (CeIII [OH]2OOH or CeIV[OH]3OOH) precipitates detectable with reflectance methods (Robinson and Batten, 1990; Van Norden and Frederiks, 1993; Halbhuber et al., 1996; Bestwick et al., 1997; Telek et al., 1999, 2001).

Fluorescent image acquisition and processing

We measured the JC-1 fluorescence signal and the cerium-ROS precipitate reflectance intensities in fixed samples. Experiments were performed in a darkened room at room temperature. A BIO-RAD MRC 1024 confocal system (Bio-Rad Corp., Hertfordshire, England) was used installed on a Nikon OPTIPHOT inverted microscope (Donsanto Corp., Natick, Massachusetts). Imaging of JC-1-labeled cells was performed using multichannel detection in fluorescence mode excitation with 488 line of a Krypton–Argon laser; standard filter set: T1, T2A). Imaging of cerium-labeled cells was performed using a single channel detection in reflectance mode, excitation with the 488 line of a Krypton–Argon laser; standard filter set (T1, T2A). Exposures, laser intensities and acquisition parameters were set the same during all acquisitions (across the groups being compared). Routine observations were carried out by simultaneously generating a green and a red fluorescent image (at 510 to 625 nm emission). For quantitative analysis high-resolution (100×) images were taken followed by a digital superposition.

Data analysis and statistics

The quantitative analysis of fluorescent JC-1 monomer (green fluorescence), J-aggregate (red fluorescence) and cerium
reflectance present in the cells was performed using a built in evaluation software LaserSharp Processing (Bio-Rad Corp., Hertfordshire, England. The fluorescence (red and green) (or reflectance) intensity was averaged within the selected cells. The ratio of red and green fluorescence was captured. In case of the cerium reflectance method the average intensity data were processed. The data were statistically evaluated using ANOVA and a post hoc Duncan test. The results were considered to be statistically significant if \( p < 0.05 \). The postcaputre analysis was the same in all cases. Data are shown as mean \pm SEM.

Results

There was no visually discernible difference in images of JC-1-stained partially neuronally differentiated PC12 cells before and after fixation with 0.25% (vol/vol) glutaraldehyde solution for 2 min. However, the postfixation JC-1 red/green pixel intensity ratios are approximately twice as big as the prefixation intensity ratios [1.57 \pm 0.19 (n = 15) versus 0.66 \pm 0.06 (n = 15)].

The partial \( O_2 \) pressure in the cell culture medium was 154.65 \pm 1.35 mm Hg (n = 6) in normoxic conditions and it was 131.96 \pm 2.29 mm Hg (n = 6) (mean \pm SEM) following the hypoxic period. Right after 1 h of hypoxia and 24 h of re-oxygenation the percentage of propidium iodide-positive (PI+) cells increased as compared to the normoxic control (36.66 \pm 3.25\% (n = 12) versus (PI+: 19.5 \pm 2.18\% (n = 12) (p < 0.001). The JC-1 red/green ratios of the cells decreased and were relatively uniform (0.681 \pm 0.013 (n = 50) versus 1.07 \pm 0.18 (n = 50) (p < 0.01). They also showed an increased cytoplasmic levels of peroxyl radicals (28.99 \pm 0.65 (n = 100) versus 23.78 \pm 0.78 (n = 100) (p < 0.001)) as measured with the cerium reflectance method.

Treatment with (-)deprenyl decreased the percentage of PI+ cells in oxygen-deprived and reperfused PC12 cell cultures at

![Fig. 1. Right after 1 h of hypoxia and 24 h of re-oxygenation the percentage of propidium iodide-positive (PI+) cells increased as compared to the normoxic control (36.66 \pm 3.25\% (n = 12) versus (PI+: 19.5 \pm 2.18\% (n = 12) (p < 0.001). Treatment with (-)deprenyl decreased the percentage of PI+ cells in oxygen-deprived and reperfused PC12 cell cultures at 10^{-12} \text{M} (21.41 \pm 3.97\% (n = 12) (p < 0.001) and 10^{-8} \text{M} (21.54 \pm 2.33\% (n = 12) (p < 0.001) significantly. 10^{-3} \text{M} (-)deprenyl, the highest concentration, increased the percentage of PI+ cells (44.25 \pm 5.76\% (n = 12) (p > 0.05) as compared to the hypoxic control (36.66 \pm 3.25\%) (p < 0.01) (Fig. 1).]

![Fig. 2. Right after 1 h of hypoxia and 24 h of re-oxygenation the cells had decreased and relatively uniform JC-1 red/green ratios 0.681 \pm 0.013 (n = 50) versus 1.07 \pm 0.18 (n = 50) (p < 0.01). The average JC-1 red/green signal intensity ratio was 0.68 \pm 0.013 (n = 50) in hypoxic control cultures, 2.83 \pm 0.1 (n = 50) (p < 0.001) at 10^{-12} \text{M}, 2.33 \pm 0.074 (n = 50) (p < 0.001) at 10^{-8} \text{M} and 1.68 \pm 0.095 (n = 50) (p < 0.01) at 10^{-3} \text{M}. The data were statistically evaluated using ANOVA and a post hoc Duncan test (* = p < 0.05; ** = p < 0.01; *** = p < 0.001). (N = normoxia; H = hypoxia; H+10^{-12} \text{M} = hypoxia+10^{-12} \text{M} (-)deprenyl; H+10^{-8} \text{M} = hypoxia+10^{-8} \text{M} (-)deprenyl; H+10^{-3} \text{M} = hypoxia+10^{-3} \text{M} (-)deprenyl).]

![Fig. 3. Right after 1 h of hypoxia and 24 h of re-oxygenation the cells showed an increased cytoplasmic levels of peroxyl radicals 28.99 \pm 0.65 (n = 100) versus 23.78 \pm 0.78 (n = 100) (p < 0.001) measured with the cerium reflectance method. The cerium signal intensity in oxygen deprived/re-oxygenated PC12 cultures was changing concentration dependently. At 10^{-12} \text{M} (20.84 \pm 0.58 (n = 100) (p < 0.001) (-)deprenyl treatment decreased the cerium signal level in hypoxic/re-oxygenated PC12 cultures below that of the normoxic control (23.78 \pm 0.78 (n = 100), at 10^{-8} \text{M} (28.86 \pm 0.78) (n = 100) (-)deprenyl did not affect it, and at 10^{-3} \text{M} (32.15 \pm 0.75) (n = 100) (p < 0.01) (-)deprenyl considerably increased it as compared to the hypoxic control (28.99 \pm 0.65) (n = 100). The data were statistically evaluated using ANOVA and a post hoc Duncan test (* = p < 0.05; ** = p < 0.01; *** = p < 0.001). (N = normoxia; H = hypoxia; H+10^{-12} \text{M} = hypoxia+10^{-12} \text{M} (-)deprenyl; H+10^{-8} \text{M} = hypoxia+10^{-8} \text{M} (-)deprenyl; H+10^{-3} \text{M} = hypoxia+10^{-3} \text{M} (-)deprenyl).]
Fig. 4. Partially neuronally differentiated, normoxic PC12 cells stained with JC1. Red color denotes active mitochondria, green color denotes cytoplasm. Imaging of JC-1-labeled cells was performed using multichannel detection in fluorescence mode excitation with 488 line of a Krypton–Argon laser; standard filter set: T1, T2A; 40× lens; image acquisition mode: simultaneous double label+accumulate to peak. Bar equals 5 μm.

The cerium signal intensity in oxygen deprived/re-oxygenated cultures was elevated by (−)deprenyl treatment in a concentration dependent fashion. The concentration of 10⁻¹² M was the most potent membrane potential booster and the 10⁻⁷ M was the least potent. The average JC-1 red/green signal intensity ratio was 0.68 ± 0.013 (n = 50) in hypoxic control cultures, 2.83 ± 0.1 (n = 50) (p < 0.001) at 10⁻¹² M, 2.33 ± 0.074 (n = 50) (p < 0.001) at 10⁻⁸ M and 1.68 ± 0.095 (n = 50) (p < 0.001) at 10⁻³ M (Figs. 2, 4, and 5).

The cerium signal intensity in oxygen deprived/re-oxygenated PC12 cultures also changed in a concentration dependent fashion. (−)Deprenyl at 10⁻¹² M (20.84 ± 0.58) (n = 100) (p < 0.001) decreased the cerium signal level in hypoxic/re-oxygenated PC12 cultures below that of the normoxic control (23.78 ± 0.78) (n = 100). On the other hand, (−)deprenyl at 10⁻⁸ M (28.86 ± 0.78) (n = 100) did not affect the level of cerium signal, while at 10⁻³ M (32.15 ± 0.75) (n = 100) (p < 0.01) it considerably increased cerium reflectance intensities as compared to the hypoxic control (28.99 ± 0.65) (n = 100) (Fig. 3).

In the normoxic control cells treated with (−)deprenyl the percentage of PI+ cells was not affected at 10⁻⁸ M (19.67 ± 2.82%) (n = 12). The concentration of 10⁻¹² M, however, considerably decreased the percentage of PI+ cells (10.18 ± 0.79%) (n = 12) and slightly decreased it at 10⁻³ M (14.83 ± 1.06%) (p > 0.05) (n = 12) as compared to the normoxic control (19.5 ± 2.18%) (n = 12). JC-1 red/green pixel intensity ratio was significantly increased in (−)deprenyl-treated normoxic control PC12 cultures at all (−)deprenyl concentrations. It was 1.07 ± 0.025 (n = 50) in normoxic control cultures, 2.15 ± 0.088 (n = 50) (p < 0.001) at 10⁻¹² M, 2.17 ± 0.077 (n = 50) (p < 0.001) at 10⁻⁸ M and 2.83 ± 0.18 (n = 50) (p < 0.001) at 10⁻³ M.

The cerium signal level, however, in the same (−)deprenyl-treated normoxic PC12 cultures was the lowest at 10⁻⁸ M (16.76 ± 1.11) (n = 100) (p < 0.001). At 10⁻³ M of (−)deprenyl concentration it was slightly increased (26.86 ± 1.05) (n = 100) (p < 0.05), while at 10⁻¹² M (24.63 ± 0.93) (n = 100) it was at the level of normoxic absolute control (23.78 ± 0.78) (n = 100). Data are summarized in Table 1.

**Discussion**

In the present study we demonstrated that the combination of the methods propidium iodide staining (for the assessment of cell death), modified JC-1 staining method (for the characterization of mitochondrial membrane potential) and cerium staining (for the in situ quantitative measurement of free radical production) provide us with a useful data set to evaluate the cytoprotective features of a selected drug. In (−)deprenyl-treated hypoxic and normoxic PC12 cell cultures evaluated by these assays a dose dependent protective effect has been found. The sensitivity of this combination of assays was such that significant effects could be detected with doses as low as 10⁻¹² M.

The present study using this combination of stainings has also demonstrated that (−)deprenyl protects cells from hypoxia/re-oxygenization, maintains mitochondrial membrane potential and prevents increases in the amounts of ROS

Fig. 5. Picture A: normoxic control (Mitochondrial polarization is indicated by an increase in the red/green fluorescence intensity ratio. Red overrides green.); picture B: hypoxia/re-oxygenation control (Mitochondrial depolarization is indicated by a decrease in the red/green fluorescence intensity ratio. Green overrides red.); picture C: hypoxia/re-oxygenization + 10⁻¹² M (−)deprenyl. Red: active mitochondria are present within the cell. Green: non-functional mitochondria are present within the cell. Imaging of JC-1-labeled cells was performed using multichannel detection in fluorescence mode excitation with 488 line of a Krypton–Argon laser; standard filter set: T1, T2A; 100× lens; image acquisition mode: simultaneous double label. Bar equals 5 μm.
induced by hypoxia/re-oxygenization in a dose-dependent manner.

In PC12 cell cultures $\Delta \Psi_M$ progressively shifted across mitochondrial populations to lower values following hypoxia/re-oxygenation and as a consequence of this shift all the cells had decreased and relatively uniform $\Delta \Psi_M$ levels. The same phenomenon was observed in heart-derived H9c2 cells following hypoxia (Kim et al., 2003), in hypothalamic neurons following hypoxia (Wu et al., 2004), and in primary rat hippocampal cultures following oxygen–glucose deprivation (Iijima et al., 2003). The re-oxygenation considerably increased the amount of intracellular ROS generated in PC12 cell cultures as observed by other authors as well (Yermolaieva et al., 2004). Following 24 h of treatment with various concentrations of (--)deprenyl during the re-oxygenation period the percentage of PI+ cells was decreased, the overall decrease in $\Delta \Psi_M$ caused by an oxygen deprivation was reversed and the perox radical levels were decreased except for the concentration of $10^{-3}$ M, revealing a U-shaped curve. Normoxic PC12 cell cultures treated with various concentrations of (--)deprenyl showed the presence of very low percentages of PI+ cells. The mitochondrial $\Delta \Psi_M$ was found to be increased at all (--)deprenyl concentrations, and the free radical level was decreased at $10^{-12}$ M.

Our data are consistent with an earlier work by Wadia and her colleagues (Wadia et al., 1998), who found that (--)deprenyl at $10^{-9}$ M concentration maintains mitochondrial membrane potential and reduces apoptosis by inducing new protein synthesis.

Since changes in mitochondrial and cellular function as a result of hypoxia/re-oxygenization have been implicated in the cascades leading to cell death, changes in $\Delta \Psi_M$ were monitored by using the potentiometric, fluorescent dye JC-1. The mitochondrial membrane potential ($\Delta \Psi_M$) controls not only ATP synthesis, mitochondrial Ca$^{2+}$ accumulation, and redox poise but also ROS generation.

Oxidative stress/ROS generation is a common detrimental factor in many different forms of neurodegenerative disease, or in hypoxia/reperfusion conditions (Yermolaieva et al., 2004). The present cerium staining used for the in situ detection of intracellular ROS is the adaptation of previous methods used by Robinson and Batten (1990), Van Norden and Frederiks (1993), Halbhuber et al. (1996), Bestwick et al. (1997) and Telek et al. (1999, 2001). Its limitation, however, similarly to all the available assays, that it is not rigorously quantitative, since the dye competes with other molecules for the interaction with ROS. The rate of oxidation of such probes shows the steady-state concentration, not the rate of production.

On the basis of their observations, Thyffault and his colleagues (1997) deducted that (--)deprenyl enhances O$_2$ formation by altering the rate of electron transfer within the respiratory chain leading to increases in SOD activities. The double nature of this drug involves the induction of an increase in mitochondrial transmembrane potential as well as elimination of free radicals at low concentrations, but at extremely high concentrations it elevates mitochondrial transmembrane potential to a harmful level resulting in the overproduction of ROS (Thyffault et al., 1997). This result, however, is only partially supported by our observations because in our experiments (--)deprenyl elevated mitochondrial transmembrane potential maximally at the concentration of $10^{-12}$ M, and the maximal intracellular ROS production was found at the concentration of $10^{-3}$ M.

### Table 2

(--)Deprenyl studies in animal stroke models

<table>
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<tr>
<th>Study</th>
<th>Species</th>
<th>Stroke model</th>
<th>Dose range</th>
<th>Study design</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jolkkonen et al., 2000</td>
<td>Rat</td>
<td>72 h of transient cerebral artery occlusion</td>
<td>0.3 mg/kg</td>
<td>Single dose following operation, as a post-treatment</td>
<td>Non-significant decrease in infarct size</td>
</tr>
<tr>
<td>Semkova et al., 1996</td>
<td>Rat</td>
<td>Permanent middle cerebral artery occlusion</td>
<td>15 mg</td>
<td>Single daily doses for 8 consecutive days as a pretreatment</td>
<td>Significant decrease in infarct size</td>
</tr>
<tr>
<td>Simon et al., 2001</td>
<td>Rat</td>
<td>Permanent middle cerebral artery occlusion</td>
<td>0.2 mg/kg/day</td>
<td>0.2 mg/kg/day continuously, for 3 days using an intraperitoneal minipump as a post-treatment</td>
<td>Significant decrease in infarct size</td>
</tr>
<tr>
<td>Unal et al., 2001</td>
<td>Mice</td>
<td>30 min middle cerebral artery occlusion and 72 h reperfusion</td>
<td>10 mg/kg</td>
<td>Daily doses for 10–14 days as a pretreatment</td>
<td>Non-significant, dose dependent effect</td>
</tr>
<tr>
<td>Erdö et al., 2000</td>
<td>Gerbil</td>
<td>Transient global cerebral ischemia</td>
<td>0.0001 mg/kg, 0.0001 mg/kg, 0.01 mg/kg</td>
<td>i.p. 6 times in 4 days following termination of ischemia</td>
<td>Significant, dose dependent effect</td>
</tr>
</tbody>
</table>
Deprenyl prevents ROS production in various in vitro and in vivo model systems (Wu et al., 1993, 1996; Khaldy et al., 2000). In our model, this unique antioxidant nature of deprenyl was associated with maintenance/boost of mitochondrial membrane potential. High ΔΨM increases the generation of ROS and maintains the reduced environment (Nicholls and Budd, 2000). Deprenyl, however, at 10⁻¹² M increases ΔΨM and decreases ROS production at the same time. Deprenyl balances the positive side of increased ΔΨM (increased mitochondrial activity) against the negative side of increased ΔΨM (increased ROS production).

The increased ΔΨM associated with a decrease in ROS levels is the possible basis of deprenyl’s anti-apoptotic (Magyar and Szende, 2000) and pro-longevity effect (Milgram et al., 1991; Knoll, 1988). Deprenyl may create the energetical basis and a comparatively peroxo radical free environment for penumbral plasticity processes following middle cerebral artery occlusion in rats (Puurunen et al., 2001; Simon et al., 2001), and following a stroke event in humans (Sivenius et al., 2001). Deprenyl may also exert a significant protective effect against memory deficits and lipid hyperperoxidation observed after cerebral ischemia (Maia et al., 2004).

However, previous studies on animal models indicate that deprenyl does not necessarily reduce the lesion size in occlusion models of brain ischemia (Jolkkonen et al., 2000; Semkova et al., 1996; Simon et al., 2001; Ünal et al., 2001; Erdő et al., 2000) (Table 2). The results of these studies suggest a need for further experiments.

If we look at the overall picture, cytoprotection was achieved at 10⁻¹² and 10⁻⁸ M concentrations of deprenyl, mitochondrial membrane protection was increased over control levels at 10⁻¹², 10⁻⁸ and 10⁻³ M concentrations and reduction of ROS was achieved only at 10⁻¹² M. In other words there seems to be no correlation between these three parameters. Deprenyl caused a significant inhibition of apoptosis in a concentration range of 10⁻⁷ to 10⁻¹⁵ M in A₁-2058 human melanoma cell culture induced by serum deprivation, while treatment with 10⁻³ M deprenyl resulted in 50% apoptosis 72 h after treatment. This biphasic effect of deprenyl on cell death was in parallel with changes in caspase-3 activity. When deprenyl was used in a high concentration (10⁻³ M) without serum deprivation, the caspase-3 activity was raised by 5 to 6 times, while low concentrations (10⁻⁷ to 10⁻⁹ M) of the drug inhibited caspase-3 activity induced by serum deprivation (Magyar and Szende, 2004). Deprenyl seems to be cytoprotective in the dose range not activating caspase-3.

Conclusion

Our study demonstrates a persistent respiratory defect of PC12 cell cultures during re-oxygenation after hypoxia. The defect is associated with lack or heavy delay of recovery of ΔΨM and reflected mostly by mitochondria progressing probably to MPT or cytochrome c release (cell death) and by increased free radical production. This mitochondrial lesion is prevented and is totally reversed by supplementation with deprenyl decreasing intracellular ROS levels and mitochondrial susceptibility to damage at 10⁻¹² M, while at 10⁻³ M promoting cell death and ROS production, showing thus a characteristic biphasic action. Because mitochondrial defects generated by hypoxia/reperfusion can cause irreversible cell injury, deprenyl treatment merits serious reconsideration for rescuing tissues injured by ischemia/reperfusion and related insults.

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