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Neuroprotective and neurotoxic effects of monoamine oxidase-B inhibitors and derived metabolites under ischemia in PC12 cells

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Abstract

Selegiline and rasagiline are selective and irreversible monoamine oxidase-B inhibitors that exert neuroprotective effects in various preclinical models. The aim of the present study was to examine the effect of selegiline and its major metabolite, L-methamphetamine in comparison to rasagiline and its major metabolite, 1-*R*-aminoindan on oxygen–glucose deprivation induced cell death in nerve growth factor (NGF)-differentiated pheochromocytoma (PC12) cells. Our results show that selegiline reduces oxygen–glucose deprivation induced cell death by 30%. When the cultures were treated with rasagiline at similar concentrations, cell death induced by oxygen–glucose deprivation was reduced by 45–55%. L-methamphetamine, a major selegiline metabolite, but not 1-*R*-aminoindan, the major rasagiline metabolite, enhanced oxygen–glucose deprivation-induced cell death by 70%. Under normoxic conditions, both metabolites lack neurotoxicity. Concomitant exposure of the cultures under oxygen–glucose deprivation, to a combination of either selegiline and L-methamphetamine or rasagiline and 1-*R*-aminoindan, indicated that L-methamphetamine, but not 1-*R*-aminoindan, blocked the neuroprotective effect of the parental drug. These results suggest there may be a neuroprotective advantage of rasagiline over selegiline.

Keywords: Parkinson's disease; Rasagiline; Selegiline; 1-*R*-aminoindan; L-methamphetamine; Neurotoxicity; Neuroprotection; Oxygen–glucose deprivation

1. Introduction

Selegiline (L-deprenyl;phenyl-isopropyl-methyl-propargylamine), a selective and irreversible inhibitor of monoamine oxidase-B, increases the efficacy of levodopa therapy in the treatment of Parkinson's disease (Birkmayer et al., 1975) by reducing dopamine catabolism by monoamine oxidase-B. Selegiline, the only monoamine oxidase-B inhibitor in clinical use to date, delayed the emergence of disability and the progression of signs and symptoms in the early phase of the disease (Parkinson's Study Group 1989, 1993). It is debatable whether the benefits associated with selegiline treatment in Parkinson's disease are due to putative neuroprotection or to the symptomatic effects of the drug. *In vivo*, selegiline is metabolized to desmethylselegiline but its major metabolites are L-amphetamine and L-methamphetamine (Heinonen et al., 1994). Amphetamine-like metabolites are neurotoxic to the brain and may cause cell damage (Oh et al., 1994). Therefore, monoamine oxidase-B inhibitors without amphetamine-like metabolites may have an advantage over selegiline in the treatment of Parkinson's disease.

Rasagiline (*N*-propargyl-1-*R*-aminoindan) is a novel, potent, selective and irreversible inhibitor of monoamine oxidase-B (Finberg et al., 1996) currently in phase III clinical trials for the treatment of Parkinson's disease. It was recently shown to protect nerve growth factor (NGF)-differentiated cells against oxygen–glucose deprivation induced cell death and to inhibit prostaglandin PGE_2 release from PC12 cells by mechanism(s) unrelated to the inhibition of monoamine oxidase-B activity (Abu-Raya et al., 1999). The major metabolite of rasagiline is 1-*R*-aminoindan (1-*R*-

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AI), which lacks amphetamine-like activities (Speiser et al., 1998).

The rat pheochromocytoma cell line PC12 (Greene and Tischler, 1976) represents a useful catecholaminergic system. After treatment with NGF for 7–14 days, PC12 cells develop features of mature sympathetic neurons, i.e., they stop dividing, develop neurite outgrowths and electrical excitability (Rudkin et al., 1989). Undifferentiated or NGF-differentiated PC12 cells have been used in neurochemistry as a model of neuronal cell death caused by a variety of insults (Mesner et al., 1992; Abu-Raya et al., 1993; Boniece and Wagner, 1993; Ferrari et al., 1993; Farinelli et al., 1996; Edsall et al., 1997; Ellren-Kashi et al., 1997; Yun et al., 1998). The above studies show that the PC12 model is a valuable cellular tool for investigating the mechanisms involved in neurotoxicity and for the development of neuroprotective drugs.

Recently, we developed a unique, novel in vitro model of oxygen–glucose deprivation i.e., ischemic-like conditions, using NGF-differentiated PC12 cultures (Abu-Raya et al., 1999, 2000). In this model, the oxygen–glucose deprivation insult results in a reduction in ATP content, activation of the arachidonic acid cascade, and cell death, typical changes of ischemic conditions. In this model, rasagiline protects from oxygen–glucose deprivation induced cell death in a dose-dependent manner (Abu-Raya et al., 1999, 2000).

The aim of the present study was to compare the neurotoxic and neuroprotective effects of rasagiline and selegiline and their major metabolites in the NGF-differentiated PC12 neuronal model under normoxia and oxygen–glucose deprivation conditions.

2. Materials and methods

2.1. Materials

The following drugs and chemicals were used in this study: lactate dehydrogenase (LDH) diagnostic kit, ascorbic acid, prostaglandin PGE_2, dextran, charcoal (Sigma, St. Louis, MO); rasagiline mesylate (TVP-1012), 1-*R*-aminoindan, selegiline (L-deprenyl), L-methamphetamine (Teva Pharmaceuticals, Petah Tikva, Israel); [^{3}H]prostaglandin PGE_2 (120–200 Ci/mmole) New England Nuclear (Boston, MA), [^{3}H]noradrenaline(36 Ci/mmole) (Amersham, Oakville, Canada); anti- prostaglandin PGE_2 antibody (BioYeda, Rehovot, Israel); NGF (Alomone Labs, Jerusalem, Israel).

2.2. PC12 cultures

PC12 cells were grown in Dulbeco's modified Eagle's medium (DMEM), supplemented with 7% fetal calf serum, 7% horse serum, 100 μg/ml streptomycin and 100 U/ml penicillin (Beit Haemek, Israel). The cultures were maintained in an incubator at 37 °C in a high humidity atmosphere of 6% CO_2. The medium was changed twice weekly and the cultures were split at a 1:6 ratio once a week (Abu-Raya et al., 1993). In the ischemic experiments, an identical number of cells ($1–1.5 \times 10^5$) was plated on 35-mm diameter petri dishes (Nunclon Delta, NUN, Denmark) coated with collagen (0.1 mg/ml). Differentiation of the cultures was induced by treatment with NGF (50 ng/ml), added every 48 h for a period of 8–10 days (Abu-Raya et al., 1999).

2.3. The ischemic device

To induce the oxygen–glucose deprivation insult, NGF-differentiated PC12 cells were introduced into an ischemic device, as previously described (Abu-Raya et al., 1993, 1999). The ischemic device is composed of two connected chambers, maintained at 37 °C by circulating hot water, in which petri dishes with NGF-differentiated PC12 cells are placed. The air in the chambers is replaced with a mixture of 95% N_2 and 5% CO_2 bubbled from a tank through a cylinder containing water, and passed through the system at a reduced flow rate to prevent evaporation of the medium (Abu-Raya et al., 1993, 1999). The oxygen level within the device is 0% (as measured online by an oxygen analyzer), representing an anoxic insult. During the experiment, glucose-free DMEM is used, and therefore, the insult is composed of both oxygen and glucose deprivation, i.e., an ischemic-like condition.

2.3.1. The ischemic paradigm

On the day of the experiment, the regular high-glucose DMEM (4.5 mg/ml) medium was removed and replaced with glucose-free, NGF (50 ng/ml) and serum (14%) containing DMEM. The cultures were then introduced into the ischemic device for about 3–4 h. At the end of the oxygen–glucose deprivation period, glucose (4.5 mg/ml) was added and the cultures were returned to normal conditions (reoxygenation) for an additional 18 h. Control cultures were maintained in the incubator under normal conditions (normoxia). In the oxygen–glucose deprivation experiments, the compounds to be tested were added to the culture medium concomitant with the oxygen–glucose deprivation insult and remained there during the reoxygenation period.

2.4. Cell death (neurotoxicity)

Cell death was measured after reoxygenation by the leakage of lactate dehydrogenase enzyme (LDH) into the medium (Abu-Raya et al., 1999), using a Sigma Diagnostics LD-L assay kit. Lactate dehydrogenase activity was determined spectrophotometrically at 340 nm by following the rate of conversion of oxidized (NAD^+) to reduced (NADH) nicotinamide adenine dinucleotide. LDH that is detected in the medium under normoxic conditions (basal LDH release) was subtracted from all LDH values obtained after oxygen–glucose deprivation, in order to get the net effect of oxygen–glucose deprivation on LDH release. Cell death after

3–4 h of oxygen–glucose deprivation without addition of the tested compounds (20–50% of total cellular LDH content) was considered as 100% cell death. Cultures exposed to oxygen–glucose deprivation after treatment with the tested compounds were compared to this 100% value.

2.5. Prostaglandin PGE_2 release

The amount of the prostaglandin PGE_2 released into the medium was determined by radioimmunoassay (RIA) as described (Abu-Raya et al., 1993). In brief, the extracellular medium was collected after reoxygenation, centrifuged at 4 °C for 10 min at 1000 × *g*, and aliquots were removed for radioimmunoassay. Following 18–24 h incubation of samples and standards with antiserum and radioligand, free and bound compounds were separated by dextran coated with activated charcoal and the radioactivity in the supernatant was measured. The basal control release of prostaglandin PGE_2 by PC12 culture is variable and range from 100 to 1000 pg/ml depending on the number of passages in culture (age of the culture) and the duration of time the individual culture is maintained and processed till the experiment.

2.6. [^{3}H]Noradrenaline release

Noradrenaline release from PC12 cells was determined with slight modifications, as previously described (Nikodijevic et al., 1990). Briefly, fresh medium was added and the cells were allowed to equilibrate at 37 °C for 30 min. The cells were then loaded with [^{3}H]noradrenaline (0.5 μCi/ml) for 3 h at 37 °C. The medium was removed and the cells were washed once with serum-supplemented medium and twice with serum-free medium containing 1 mM ascorbic acid. Fresh medium was added, and the cultures were exposed to oxygen–glucose deprivation conditions for 2 h in the presence or absence of L-methamphetamine. Basal release was measured in cultures incubated under normoxic conditions. At the end of the oxygen–glucose deprivation period, 0.2-ml sample was removed from the cultures medium, centrifuged for 10 min (1000 × *g*) to remove floating cells, and the radioactivity was measured in a liquid scintillation counter.

2.7. Analysis of methamphetamines and amphetamine

NGF-differentiated PC12 cultures were treated with selegiline (1 μM) and then exposed to oxygen–glucose deprivation conditions for 3 h followed by reoxygenation for 0, 3, or 18 h. The cells were then scraped into the incubation medium and the mixture was transferred to Eppendorf tubes and frozen at −80 °C. Gas chromatography with electron capture detection (GC-ECD) was applied for the analysis of methamphetamine (L-METH) and amphetamine: the samples were thawed, disrupted using a sonicator, and extracted with hexane after the addition of base and internal standard. The amine in the hexane layer was derivatized with trichloroacetyl chloride. After a cleaning procedure, the samples were analyzed by gas chromatography using an electron capture detection detector and a capillary column. Calibration curves ranging from 10 to 100 ng/ml for methamphetamine and amphetamine were established. Specificity was demonstrated by running two control cell samples.

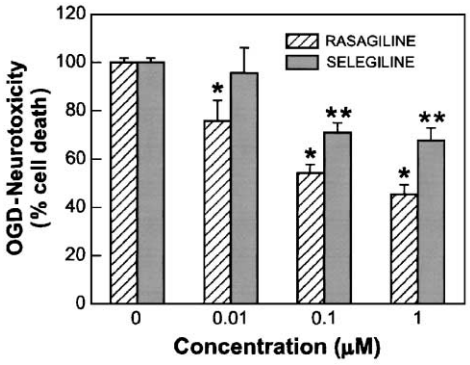

Fig. 1. The effect of selegiline and rasagiline on oxygen–glucose deprivation (OGD)-induced cell death. PC12 cultures differentiated with NGF (50 ng/ml) for 8–10 days and were then exposed to oxygen–glucose deprivation for 3 h followed by reoxygenation for 18 h. Cultures were treated with several concentrations of selegiline or rasagiline (added upon initiation of the insult) or left untreated. At the end of the reoxygenation period, cell death was measured by LDH release in to the medium. In rasagiline- or selegiline-treated culture, cell death was expressed as % of the value obtained in non-treated cultures (oxygen–glucose deprivation alone, considered 100%, see Materials and methods). The values presented are the mean ± S.E.M. of three independent experiments ($n = 3$). * $P < 0.05$, * * $P < 0.05$, vs. no treatment (ANOVA). The effect of rasagiline compared to selegiline was significant at 0.1 and 1 μM, $P < 0.05$ (Student's *t*-test).

2.8. Statistics

The results are presented as the mean ± S.E.M. of several experiments performed in triplicate. Statistical analysis of variance and the significance of differences between experimental groups was performed using analysis of variance program (ANOVA), and was considered significant when *P* values < 0.05 were obtained. The comparison between the neuroprotective effect of rasagiline and selegiline presented in Fig. 1 was statistically analysed by Student's paired *t*-test. *P* values of $P < 0.05$ were considered statistically significant.

3. Results

NGF-differentiated PC12 cells were treated with several concentrations of rasagiline or selegiline at the start of the oxygen–glucose deprivation insult for 3 h followed by reoxygenation for 18 h. Cell death was measured after the reoxygenation period. As shown in Fig. 1, rasagiline reduced oxygen–glucose deprivation-induced cell death by about 25%, 45%, and 55% at 0.01, 0.1 and 1 μM, respectively. As can be seen in Fig. 1, at 0.01 μM selegiline did not

affect cell death induced by oxygen–glucose deprivation. However, at 0.1 and 1 μM, it reduced oxygen–glucose deprivation-induced cell death by about 30%. This indicates that rasagiline protects NGF-differentiated PC12 cells from oxygen–glucose deprivation-induced cell death to a greater extent than does selegiline.

In vivo selegiline is metabolized into L-amphetamine, L-methamphetamine and desmethylselegiline. L-methamphetamine accounts for the majority of the metabolites (Heinonen et al., 1994). To test the effect of L-methamphetamine on oxygen–glucose deprivation-induced cell death, NGF-differentiated PC12 cells were treated with several concentrations of L-methamphetamine and exposed to oxygen–glucose deprivation followed by reoxygenation. As shown in Fig. 2, up to 1 μM of L-methamphetamine caused no significant neurotoxicity. L-methamphetamine at 1 and 10 μM enhanced oxygen–glucose deprivation-induced cell death by about 70%. To test the effect of L-methamphetamine on cell death under normal conditions, NGF-differentiated PC12 cultures were treated with several concentrations of L-methamphetamine and the cultures were incubated for 18 h. In untreated cultures, basal LDH release was about 6% of the total content. In L-methamphetamine treated cultures, LDH release was 6%, 7%, and 8% of the total content at 1, 10, and 100 μM, respectively. These findings indicate that L-methamphetamine has a neurotoxic effect on NGF-differentiated PC12 cultures under oxygen–glucose deprivation conditions but not under conditions of normoxia. Recently, we demonstrated that there is a correlation between cell death and prostaglandin PGE_2 release in PC12 cultures exposed to toxins (Ellren-Kashi et al., 1997) and oxygen–glucose deprivation (Abu-Raya et al., 1999). Therefore, we tested the effect of L-methamphetamine on oxygen–glucose deprivation-induced prostaglandin PGE_2 release as an additional

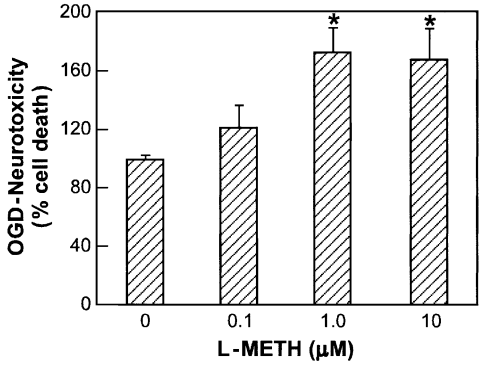

Fig. 2. The effect of L-methamphetamine (L-METH) on oxygen–glucose deprivation-induced cell death. PC12 cultures differentiated with NGF (50 ng/ml) for 8–10 days and were then exposed to oxygen–glucose deprivation for 3 h followed by reoxygenation for 18 h. The cultures were treated with L-methamphetamine at the indicated concentrations or left untreated. At the end of the reoxygenation period, cell death was measured by LDH release in to the medium. Cell death was expressed as % of the value obtained in non-treated cultures (see legend in Fig. 1 and Materials and methods). The values presented are the mean ± S.E.M. of three independent experiments ($n=3$). * $P<0.05$ vs. no treatment.

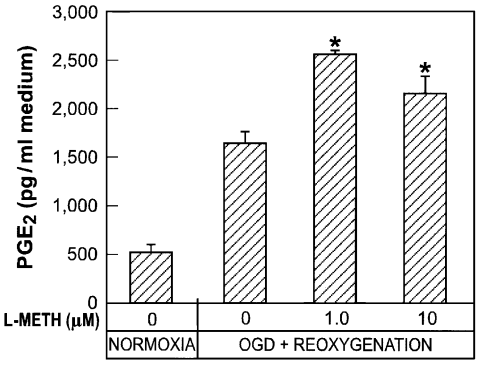

Fig. 3. The effect of L-methamphetamine (L-METH) on oxygen–glucose deprivation-induced prostaglandin PGE_2 (PGE_2) release in NGF-differentiated PC12 cells. PC12 cultures differentiated with NGF (50 ng/ml) for 8–10 days and were then exposed to oxygen–glucose deprivation for 3 h followed by reoxygenation for 18 h. Cultures were treated with L-methamphethamine (L-METH) at the indicated concentrations or left untreated. At the end of the reoxygenation period, PGE_2 released into the extracellular medium was measured by radioimmunoassay. Basal prostaglandin PGE_2 release was measured under normoxic conditions (Normoxia). The values presented are the mean ± S.E.M. of a representative experiment ($n=3$). * $P<0.05$ vs. oxygen–glucose deprivation alone.

marker of neurotoxicity. NGF-differentiated PC12 cells were treated with L-methamphetamine at concentrations enhancing oxygen–glucose deprivation-induced cell death (Fig. 2), and exposed to the oxygen–glucose deprivation insult, followed by reoxygenation for 18 h. Prostaglandin PGE_2 release was measured after the reoxygenation period. As shown in Fig. 3, oxygen–glucose deprivation increased by three-fold prostaglandin PGE_2 release into the extracellular medium, as we previously reported in undifferentiated (Abu-Raya et al., 1993) and NGF-differentiated (Abu-Raya et al., 1999) PC12 cultures. At 1 and 10 μM, L-methamphetamine enhanced oxygen–glucose deprivation-induced prostaglandin PGE_2 release by five- and four-fold, respectively (Fig. 3). We similarly tested the effect of the major metabolite of rasagiline, 1-*R*-AI, on oxygen–glucose deprivation-induced cell death and prostaglandin PGE_2 release. As shown in Fig. 4, at concentrations up to 10 μM, 1-*R*-aminoindan did not affect oxygen–glucose deprivation-induced cell death. Furthermore, 1-*R*-aminoindan had no effect on oxygen–glucose deprivation-induced prostaglandin PGE_2 release (Fig. 5). In addition, this compound was not found neurotoxic (at concentrations up to 100 μM) to the NGF-differentiated cultures under normoxic conditions (data not shown). This indicates that 1-*R*-aminoindan, in contrast to L-methamphetamine, is not toxic to NGF-differentiated PC12 cultures under oxygen–glucose deprivation conditions. It was reported that L-methamphetamine toxicity appears to be due to dopamine release, followed by oxidation of the catecholamines, and the production of hydrogen peroxide and hydroxyl radical (Lamensdorf et al., 2000). Therefore, we tested the effect of oxygen–glucose deprivation conditions on the release of noradrenaline from NGF-differentiated PC12 cultures in the presence or absence of methamphetamine. For this

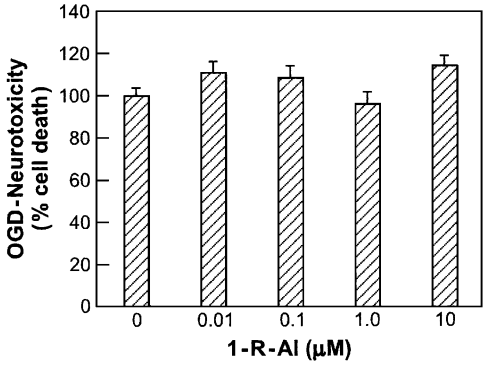

Fig. 4. The effect of 1-*R*-aminoindan (1-*R*-AI) on oxygen–glucose deprivation-induced cell death. PC12 cultures differentiated with NGF (50 ng/ml) for 8–10 days and were then exposed to oxygen–glucose deprivation for 3 h followed by reoxygenation for 18 h. Cultures were treated with 1-*R*-aminoindan at the indicated concentrations or left untreated. At the end of the reoxygenation period, cell death was measured by LDH release in to the medium. Cell death was expressed as % of the value obtained in non-treated cultures (see legend in Fig. 1 and Materials and methods). The values presented are the mean ± S.E.M. of three independent experiments ($n=3$).

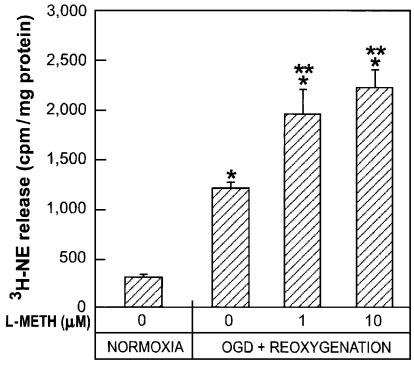

Fig. 6. The effect of oxygen–glucose deprivation and L-methamphetamine on [^{3}H]noradrenaline release from NGF-differentiated PC12 cells. PC12 cultures differentiated for 8–10 days with NGF (50 ng/ml) loaded with [^{3}H]noradrenaline were exposed to oxygen–glucose deprivation for 2 h in the presence or absence of L-methamphetamine at the indicated concentrations. The values presented are the mean ± S.E.M. of a representative experiment ($n=3$). * $P<0.05$ vs. normoxia, ** $P<0.05$ vs. oxygen–glucose deprivation alone.

purpose, NGF-differentiated PC12 cultures loaded with [^{3}H]noradrenaline were exposed to oxygen–glucose deprivation for 2 h (under these conditions minimal cell death was observed, Abu-Raya et al., 1999) in the presence or absence of L-methamphetamine. As shown in Fig. 6, oxygen–glucose deprivation alone increases by two-fold noradrenaline release compared to normoxia cultures. In cultures treated with 1 and 10 μM L-methamphetamine oxygen–glucose deprivation-induced noradrenaline release was enhanced by 63% and 67%, respectively.

We investigated the possibility that selegiline is metabolized to amphetamine-like products in NFG-differentiated PC12 cultures, as reported in animal and clinical studies. Selegiline at a concentration of 1 μM was added, and the cultures were exposed to oxygen–glucose deprivation. The cultures were then harvested, and the level of L-methamphetamine and L-amphetamine was analyzed, using the gas chromatography electron capture detection (GC-ECD) method. These major metabolites were not detected during the experiment, considering a sensitivity of 50 nM of the above bioanalytical method. This data indicates that selegi-

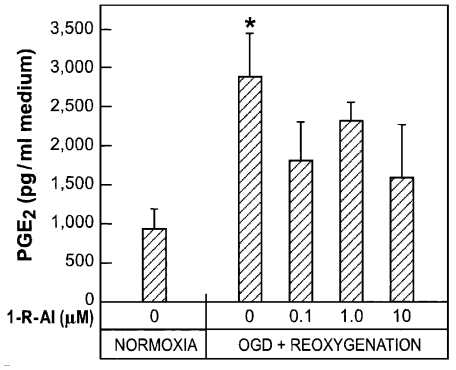

Fig. 5. The effect of 1-*R*-aminoindan (1-*R*-AI) on oxygen–glucose deprivation-induced prostaglandin PGE_2 (PGE_2) release in NGF-differentiated PC12 cells. PC12 cultures differentiated with NGF (50 ng/ml) for 8–10 days and were then exposed to oxygen–glucose deprivation for 3 h followed by reoxygenation for 18 h. The cultures were treated with 1-*R*-aminoindan or left untreated. At the end of the reoxygenation period, prostaglandin PGE_2 released into the extracellular medium was measured by radioimmunoassay. Basal PGE_2 release was measured under normoxic conditions (Normoxia). The values presented are the mean ± S.E.M. of a representative experiment ($n=3$). * $P<0.05$ vs. normoxia.

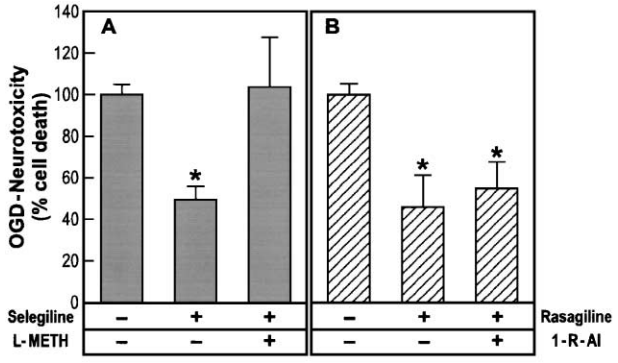

Fig. 7. The effect of L-methamphetamine (L-METH) and 1-*R*-aminoindan (1-*R*-AI) on the neuroprotective effect of selegiline (A) or rasagiline (B) on oxygen–glucose deprivation-induced cell death. PC12 cultures differentiated with NGF (50 ng/ml) for 8–10 days and were then exposed to oxygen–glucose deprivation for 3 h followed by reoxygenation for 18 h. The cultures were treated with selegiline (1 μM) with (1 μM; +; +) or without L-methamphetamine (+; –) or rasagiline (1 μM) with (1 μM; +; +) or without 1-*R*-aminoindan (+; –) at the indicated concentrations or left untreated (–; –). At the end of the reoxygenation period, cell death was measured by LDH release in to the medium. Cell death was expressed as % of the value obtained in non-treated cultures (see legend in Fig. 1 and Materials and methods). The values presented are the mean ± S.E.M. of a representative experiment ($n=3$). * $P<0.05$ vs. oxygen–glucose deprivation alone.

line is not metabolized to a significant extent to amphetamine-like compounds in NGF-differentiated PC12 cultures. In order to mimic certain in vivo pharmacological situation in which both the parental and the metabolite drugs are present and active, PC12 neuronal cultures were exposed to a combination of parental drug and the respective metabolite at the maximal effective concentration. The cultures were exposed at the start of oxygen–glucose deprivation to a combination of selegiline (1 μM) and L-methamphetamine (1 μM) (Fig. 7A) or to rasagiline (1 μM) and 1-*R*-aminoindan (1 μM) (Fig. 7B) for 3.5 h. It is evident that 1-*R*-aminoindan does not alter the neuroprotective effect of rasagiline. However, L-methamphetamine completely blocked the neuroprotective effect of selegiline.

4. Discussion

In the present study, we compared the neuroprotective effect of selegiline (deprenyl) to that of rasagiline. Our results indicate that selegiline protects NGF-differentiated PC12 cultures against oxygen–glucose deprivation-induced cell death but to a lesser extent than rasagiline. We also found that the major metabolite of selegiline, L-methamphetamine, enhances oxygen–glucose deprivation-induced cell death, as opposed to the major metabolite of rasagiline, 1-*R*-aminoindan, which does not affect cell death under these conditions. Our results also indicate a physiological antagonism between selegiline and L-methamphetamine but not between rasagiline and 1-*R*-aminoindan.

Rasagiline exerts a neuroprotective effect in several neuronal systems: reduces glutamate toxicity in cultures of hippocampal neurons (Finberg et al., 1996), increases the survival of dopaminergic neurons under serum-free conditions (Finberg et al., 1998), prevents deficits in cholinergic functions and behavior following hypoxia in adult and senescent rats (Speiser et al., 1998), protects against experimental focal ischemia in the rat (Speiser et al., 1999) and against closed head injury in the mouse (Huang et al., 1999). Using the in vitro oxygen–glucose deprivation (OGD) model of NGF-differentiated PC12 cultures (Abu-Raya et al., 1999, 2000), we found that rasagiline reduced oxygen–glucose deprivation-induced cell death in a dose-dependent manner. Rasagiline was effective also when given after the oxygen–glucose deprivation insult (Abu-Raya et al., 1999, 2000). In cultures treated with rasagiline and exposed to oxygen–glucose deprivation, there was a smaller reduction in ATP content, and a drop in oxygen–glucose deprivation-induced prostaglandin PGE_2 release, as compared with that in cultures exposed to oxygen–glucose deprivation alone (Abu-Raya et al., 1999). In the present study selegiline, at 0.1 and 1 μM, reduced oxygen–glucose deprivation-induced cell death by about 30%, compared with 55% in cultures treated with rasagiline ($P < 0.05$ as evaluated by Student's *t*-test, Fig. 1). This indicates that the efficacy of rasagiline is greater than that of selegiline, as it confers neuroprotection at lower concentrations and provides a greater level of protection at the same concentrations. It is unlikely that this effect is related to greater inhibition of monoamine oxidase-B, since NGF-differentiated PC12 cultures (Abu-Raya et al., 1999) as well as the undifferentiated cultures (Youdim et al., 1986; Youdim, 1991) contain monoamine oxidase type A rather than type B. Furthermore, clorgyline, a monoamine oxidase-A inhibitor, did not protect the cultures against oxygen–glucose deprivation-induced cell death (Abu-Raya et al., 1999, 2000). These findings support the concept that the neuroprotective effect of rasagiline and selegiline under oxygen–glucose deprivation conditions in NGF-differentiated PC12 cells is independent of monoamine oxidase inhibition. Rasagiline and selegiline are both propargylamines but rasagiline is 5–10 times more potent that selegiline as a monoamine oxidase inhibitor (Sterling et al., 1998). The neuroprotective effects of selegiline are also well documented: several preclinical studies have demonstrated that selegiline promotes, independently of monoamine oxidase-B inhibition, neuronal survival both in vivo and in vitro. Selegiline protects against the damage caused by ischemia (Knollema et al., 1995; Semkova et al., 1996; Lahtinen et al., 1997), toxicity of 1-methyl-4-phenylpyridinium (MPP^+) (Mytilineou and Cohen, 1985; Koutsilieri et al., 1994) and NMDA (Mytilineou et al., 1997), and increases the survival of fetal dopaminergic neurons (Roy and Bedard, 1993). Furthermore, selegiline enhances the survival of motoneurons after axotomy (Ansari et al., 1993) and of NGF-differentiated PC12 cells following withdrawal of NGF (Tatton et al., 1994). However, in contrast to rasagiline, selegiline is an amphetamine derivative and is metabolized to amphetamine-like compounds in brain and peripheral tissues (Heinonen et al., 1994). These metabolites, particularly L-methamphetamine, are present in human urine for as long as 7 days after dosing, indicating that L-methamphetamine is long-lived in mammalian brain (Mauer and Kraemer, 1992). The metabolites may cause undesirable side effects (Engberg et al., 1991) and, possibly, cell death (Oh et al., 1994). In the present study, the major metabolite of selegiline, L-methamphetamine, at 1 and 10 μM increased oxygen–glucose deprivation-induced cell death (Fig. 2), enhanced oxygen–glucose deprivation-induced prostaglandin PGE_2 release (Fig. 3) and reversed the neuroprotective effect of selegiline (Fig. 7). However, at concentrations up to 100 μM, L-methamphetamine did not induce cell death when the cultures were maintained under normal conditions. These results indicate that under oxygen–glucose deprivation conditions L-methamphetamine has a neurotoxic effect on NGF-differentiated PC12 cultures. Selegiline is not metabolized to any significant extent to amphetamine-like products in PC12 cells. Therefore, the reduced neuroprotective effect of selegiline in comparison with that of rasagiline cannot be attributed to the metabolism of selegiline to amphetamine-like compounds. Although NGF-differenti-

ated PC12 cultures cannot produce amphetamine-like products under oxygen–glucose deprivation conditions, they still represent a pharmacological model since we could measure a physiological antagonism between selegiline and L-methamphetamine (Fig. 7A).

It was reported that L-methamphetamine toxicity appears to be due to L-methamphetamine-induced release of catecholamine from synaptic vesicles, oxidation of the catecholamine neurotransmitter in the cytoplasm and, subsequently, hydrogen peroxide production. The hydrogen peroxide can then interact with iron and be converted to the hydroxyl radical, which, in turn, elicits axonal degeneration followed by hydrolysis of DNA, proteins and lipids (Wei et al., 1997). It is conceivable that these processes are enhanced under oxygen–glucose deprivation conditions, as hypoxia (Kumar et al., 1998; Taylor and Peers, 1998) or oxygen–glucose deprivation (Fig. 6) itself increases catecholamines release in PC12 cells. Our results indicate that under oxygen–glucose deprivation conditions the release of noradrenaline (Fig. 6) precedes LDH release (Abu-Raya et al., 1999), which raises a causal relationship between the enhancement of oxygen–glucose deprivation-induced neurotransmitter release by L-methamphetamine and cell death (Fig. 2). Other experiments have shown that treatment with protein synthesis inhibitors blocks methamphetamine-induced damage to dopaminergic neurons (Finnegan and Karler, 1992). These findings suggest that methamphetamine may cause neuronal death by stimulating "neurotoxic proteins" that mediate programmed neuronal death (Finnegan and Karler, 1992). More experiments are required to clarify the mechanism by which L-methamphetamine enhances oxygen–glucose deprivation-induced cell death. In contrast to L-methamphetamine, the rasagiline metabolite 1-*R*-aminoindan did not affect oxygen–glucose deprivation-induced cell death (Fig. 4) or prostaglandin PGE_2 release (Fig. 5), indicating that it has no toxic effect in NGF-differentiated PC12 cultures under oxygen–glucose deprivation conditions.

In conclusion, the present study shows that rasagiline is a more potent neuroprotective compound than selegiline under oxygen–glucose deprivation conditions in NGF-differentiated PC12 cultures. Furthermore, unlike the major selegiline metabolite L-methamphetamine, the rasagiline metabolite 1-*R*-aminoindan had no toxic effect under these conditions. Therefore, rasagiline may have an advantage over selegiline since its neuroprotective properties are uncomplicated by the production of neurotoxic metabolites such as amphetamine-like compounds.

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