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Mini-Review

Rasagiline: Neurodegeneration, Neuroprotection, and Mitochondrial Permeability Transition

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Mitochondria are involved directly in cell survival and death. The assumption has been made that drugs that protect mitochondrial viability and prevent apoptotic cascade-induced mitochondrial permeability transition pore (MPTp) opening will be cytoprotective. Rasagiline (*N*-propargyl-1*R*-aminoindan) is a novel, highly potent irreversible monoamine oxidase (MAO) B inhibitor anti-Parkinson drug. Unlike selegiline, it is not derived from amphetamine, and is not metabolized to neurotoxic L-methamphetamine derivative. In addition, it does not have sympathomimetic activity. Rasagiline is effective as monotherapy or adjunct to levodopa for patients with early and late Parkinson's disease (PD) and adverse events do not occur with greater frequency in subjects receiving rasagiline than in those on placebo. Phase III controlled studies indicate that it might have a disease-modifying effect in PD that may be related to its neuroprotective activity. Its *S* isomer, TVP1022, is more than 1,000 times less potent as an MAO inhibitor. Both drugs, however, have neuroprotective activity in neuronal cell cultures in response to various neurotoxins, and in vivo in response to global ischemia, neurotrauma, head injury, anoxia, etc., indicating that MAO inhibition is not a prerequisite for neuroprotection. Their neuroprotective effect has been demonstrated to be associated directly with the propargylamine moiety, which protects mitochondrial viability and MTPp by activating Bcl-2 and protein kinase C (PKC) and by downregulating the pro-apoptotic FAS and Bax protein families. Rasagiline and its derivatives also process amyloid precursor protein (APP) to the neuroprotective, neurotrophic, soluble APP α (sAPPα) by PKC- and MAP kinase-dependent activation of α-secretase. The identification of the propargylamine moiety as the neuroprotective component of rasagiline has led us to development of novel bifunctional anti-Alzheimer drugs (ladostigil) possessing cholinesterase and brain-selective MAO inhibitory activity and a similar neuroprotective mechanism of action.

Key words: rasagiline; propargyl moiety; Bcl-2 family members; protein kinase C; caspase-3; SHSY5Y neuroblastoma cells

The anti-Parkinson drug (Parkinson Study Group, 2002, 2004), rasagiline (*N*-propargyl-[1*R*]aminoindan) is a potent second-generation monoamine oxidase (MAO) B inhibitor (Youdim et al., 2001a; Finberg and Youdim, 2002). Our studies have provided new insights into the molecular mechanism of neuroprotection induced by rasagiline, its derivatives, and the *N*-propargylamine moiety against a variety of neurotoxins that open the mitochondrial permeability transition pore (MPTp). This involves the Bcl-2 protein family associated with protein kinase C (PKC) pathway activation and interaction with mitochondrial permeability transition (MPT) (Nishizuka, 1988).The latter processes are involved in neuronal survival and functions of neuronal trophic factors (Montz et al., 1985; Hama et al., 1986). They are also critical in formation and consolidation of different types of memory (Vianna et al.,

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2000), suggesting a crucial role for Bcl-2 family proteins and PKC in the aberrant signal transduction occurring in dementia of Alzheimer's disease (AD), Parkinson's disease (PD), and Lewy Body disease (DLB) (Jin and Saitoh, 1995). A deficit in PKC isoform levels in AD and PD (Masliah et al., 1991; Shimohama et al., 1993; Matsushima et al., 1996) is thought to lead to reduced responsiveness of brain tissues to growth factors and neurotransmitters, including acetylcholine and dopamine (Jin and Saitoh, 1995; Roth et al., 1995), and to increased degeneration of neurons. A defect in PKC activation in AD has been documented as a marked loss of redistribution of cytosolic PKC to the particulate fraction in response to phorbol esters and K^+ depolarization in tissue slices from hippocampus, temporal, and frontal cortex (Wang et al., 1994). In vitro and in vivo studies have demonstrated that PKC and PKC-coupled receptors are involved in neuroprotection and in the non-amyloidogenic α-secretase pathway of amyloid precursor protein (APP) processing to the neuroprotective, neurotrophic, soluble amyloid precursor protein α (sAPPα) (Buxbaum et al., 1990; Nitsch et al., 1992; Slack et al., 1993; Caputi et al., 1997; Rossner et al., 1997; Lin et al., 1999; Yogev-Falach et al., 2002, 2003; Bar-Am et al., 2004a,b). From what has been learned about neuroprotective mechanism of rasagiline, we have thus developed neuroprotective anti-Alzheimer drugs (ladostigil [TV3326] and TV3279) from the pharmacophore of rasagiline. The latter compounds similarly activate antiapoptotic Bcl-2 family proteins and PKCs. Our studies indicate that the mechanism of their neuroprotective activity involves interaction at MPT.

MOLECULAR MECHANISM OF NEUROPROTECTION BY RASAGILINE AND OTHER PROPARGYLAMINES

Accumulating evidence indicates that particular sets of neuronal cells associated with neurodegenerative diseases, such as AD and PD may die of apoptosis (Jellinger, 2000; Yuan and Yankner, 2000), but by no means has this been established fully. Because rasagiline and ladostigil have been developed as drugs possessing possible disease-modifying activity, it was essential to investigate mechanisms underlying the prevention of neuronal cell death. Neuronal cell survival mainly involves activation of PKC-mediated cell viability pathway (Maher, 2001), whereby a classic PKC associates with the Bcl-2 protein family (Ruvolo et al., 1998). The Bcl-2 family proteins are key regulators of the apoptosis program in neurons and may either support cell survival (Bcl-2, Bcl-xL, Bcl-w, Mcl-1, and A1/Bfl-1) or promote cell death (Bax, Bak, Bcl-Xs, Bad, Bid, Bik, Hrk, and Bok) (Tsujimoto and Shimizu, 2000; Cory and Adams, 2002). The members of the Bcl-2 family interact among each other to form a dynamic equilibrium between homo- and heterodimers. Because members of these opposing actions can associate and seemingly titrate each other's function, their relative abundance in a particular cell type may determine the threshold for apoptosis (Oltvai et al., 1993). The competitive action of pro- and anti-survival Bcl-2 family proteins regulates activation of the proteases (caspases) that dismantle the cell (Adams and Cory, 1998; Evan and Littlewood, 1998; Zamzami et al., 1998).

Rasagiline (Youdim et al., 2001a; Parkinson Study Group 2002, 2004) has broad neuroprotective activity against a variety of neurotoxins in neuronal cell cultures (Finberg et al., 1998; Maruyama et al., 2001b,c; Youdim et al., 2001b, 2003) and in animal models of closed head trauma (Huang et al., 1999), global and focal ischemia (Speiser et al., 1999) and *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-(MPTP)-induced neurotoxicity (Sagi et al., 2001, 2003) and transgenic mouse model of amyotrophic lateral sclerosis (Waibel et al., 2004) and 6-hydroxydopamine model of Parkinson's disease in rat (Blandini et al., 2004). The molecular mechanism of neuroprotection by rasagiline has been studied in SHSY5Y and PC12 cells in culture against apoptosis induced by *N*-methyl-*R*-salsolinol, the peroxynitrite donor SIN-1, and 6-hydroxydopamine. It has also been studied in serum and nerve growth factor (NGF) withdrawal (Naoi and Maruyama, 2001; Youdim and Weinstock, 2001; Youdim, 2003). The demonstration that its optical *S* isomer, TVP1002, which is 1,000 times less active as an MAO inhibitor, has a similar neuroprotective activity (Maruyama et al., 2000; Youdim et al., 2001b) indicates that the neuroprotective activity of rasagiline and other propargylamines are not dependent on their MAO inhibitory activity.

Rasagiline and its related propargylamines suppress the apoptotic cell death cascade initiated by mitochondria (Youdim et al., 2001b; Akao et al., 2002b). In response to neurotoxins (SIN-1 or *N*-methyl-*R*-salsolinol), they prevent preapoptotic swelling of mitochondria and the decline in mitochondrial membrane potential ($\Delta\Psi m$) resulting from permeability transition. They also prevent the following apoptotic processes: activation of caspase 3; activation of nuclear PARP-1; translocation of glyceraldehyde-3-phosphate dehydrogenase (GADPH); and nucleosomal DNA fragmentation (Youdim and Weinstock, 2001; Youdim, 2003; Bar-Am et al., 2004a,b; Weinreb et al., 2004). In addition, rasagiline increases expression of anti-apoptotic Bcl-2 and Bcl-xL and downregulates pro-apoptotic Bad and Bax in SHSY5Y cells (2001a; Youdim, 2003; Yogev-Falach et al., 2003; Bar-Am et al., 2004a,b).

RASAGILINE AND MPT

Mitochondria play a critical role in and are potent integrators and coordinators of cell death (apoptosis and necrosis) and survival. Apoptosis and necrosis are modes of cell death that play an integral part in many biological processes and their demise have been implicated in a variety of neurodegenerative and nonneurodegenerative diseases such as cardiovascular disease and diabetes (see for reviews Suleiman et al., 2001; Belzacq et al., 2002; Halestrap et al., 2002; Belzacq and Brenner, 2003). The participation of mitochondrial-induced apoptosis in neuronal cell death has not been established fully and remains a controversial subject. Nevertheless, it has been a target for study of neurotoxin-induced cell death and neuroprotection in progressive loss of neurons and cytoprotection in cardiovascular diseases with a variety of pharmacologic

agents. The initial phase of apoptosis is triggered in response to an induction phase resulting from a variety of insults, such as xenobiotics, Parkinsonism-endogenous (*N*-methyl-*R*-salsolinol) and exogenous (6-hydroxydopamine and MPTP) neurotoxins, radiation, oxidative stress, and glucose and oxygen deprivation. This is accompanied by a change in mitochondrial membrane permeability (MMP) that results in opening of the MPTp complex, a nonspecific pore, under conditions of elevated matrix Ca^{2+}, oxidative stress, and depletion of adenine nucleotides. MPTp opening causes a massive swelling of mitochondria and a decline in mitochondrial membrane potential ($\Delta\Psi$m) resulting from the rupture of the outer membrane. This, followed by inhibition of ubiquitin-proteasome complex, release of mitochondrial cytochrome c, and activation of caspases (especially caspase 3) and PARP-1, results in cell death by apoptosis (Belzacq et al., 2002, 2003; Halestrap et al., 2002). MPTp plays a central role in induction and prevention of apoptosis-induced cell death and consists of a mitochondrial multiprotein complex that includes porin, hexokinase, peripheral benzodiazepine receptor, creatine kinase, adenosine nucleotide translocase, and cyclophilin D; however, its exact nature is not yet known.

The direct involvement of MPTp in apoptotic-induced death of mammalian cells including neurons in cell culture and in vivo in variety of models has been well documented (Youdim, 2003). The important role of MPTp is also supported by the findings that the MPTp complex, particularly the voltage-dependent anion channel (VDAC) and adenosine nucleotide translocase (ANT), are direct functional targets for Bcl-2 family proteins. Mitochondrial phase function is controlled by oncogenes and antioncogenes of the Bcl-2-Bax family. Antiapoptotic members (Bcl-2, Bcl-xL, etc.) stabilize MPTp, whereas proapoptotic members (Bax, Bak, Bad, and Bid) promote and increase in MPP (see for reviews Suleiman et al., 2001; Belzcaq et al., 2002, 2003; Halestrap et al., 2002; Belzacq and Brenner, 2003). For example, we have shown that in response to the endogenous dopaminergic neurotoxin *N*-methyl-*R*-salsolinol in the integration phase, there occurs in neuroblastoma SHSY5Y cell mitochondria a loss of $\Delta\Psi$m, swelling of mitochondria matrix, oxidative stress, and opening of MPP, whereas pretreatment of the cells with rasagiline prevents these responses (Akao et al., 2002a,b). In addition, rasagiline prevents inhibition of the ubiquitin-proteasome system by the neurotoxin that results in release of toxic intermembrane proteins such as cytochrome c. These are followed by a complex set of events, which includes activation of procaspases and caspase 3, resulting in nuclear chromatin condensation, DNA fragmentation and change in plasma membrane that culminates in neuron death (Akao et al., 2002a,b).

In structural activity studies, we investigated the mechanism underlying the relation between Bcl-2 family members and PKC signaling in neuroprotection and MPT to determine the molecular structure responsible for neuroprotective activities of rasagiline and its bifunctional

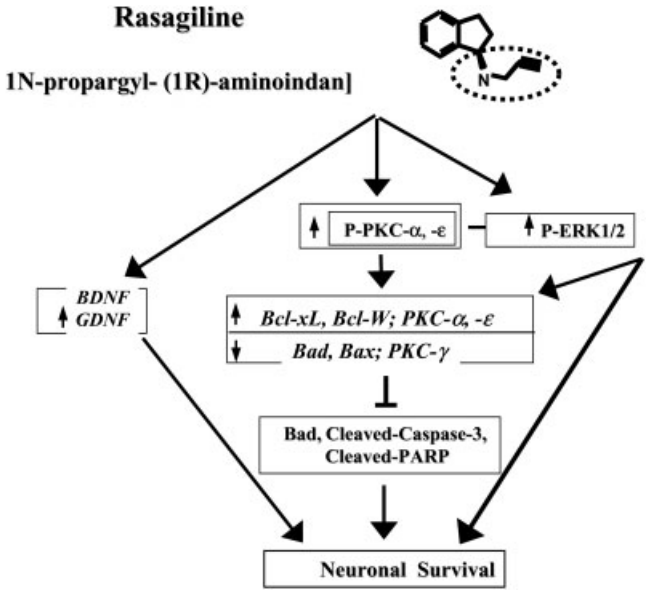

Fig. 1. Schematic representation of the mechanism of neuroprotective action of rasagiline that is also shared by ladostigil (TV3326) and *N*-propargylamine. These propargylamines activate PKCα and ε but downregulate δ and γ. In this respect, they behave opposite to pro-apoptotic drugs. They also activate ERK1/2 phosphorylation, which together with PKC have been shown to be responsible for the neuroprotective activities because PKC and ERK inhibitors prevent cell survival activity via activation of antiapoptotic Bcl-2 family proteins and downregulation of caspase 3 and cleaved PARP. At least rasagiline has also been shown to induce BDNF and GDNF and release of the latter. This may account for the neuroprotective and possible neurorescue activity of these drugs (Yogev-Falach et al., 2002, 2003; Youdim, 2003; Bar-Am et al., 2004; Maruyama et al., 2004; Weinreb et al., 2004).

derivative ladostigil (TV3326). Rasagiline-induced neuroprotection exhibited in response to the neurotoxin *N*-methyl-*R*-salsolinol is seen also with other neurotoxic events and compounds (e.g., SIN-1, glutamate, and Aβ amyloid) that induce neuronal death (Maruyama et al., 2000; Maruyama et al, 2001a,b; Yogev-Falach et al., 2003; Weinreb et al., 2004). This has provided the first evidence that propargylamines and rasagiline antiapoptotic drugs regulate the anti- and proapoptotic signaling pathways in mitochondria (Youdim, 2003). The ability of rasagiline to inhibit *N*-methyl-*R*-salsolinol-induced collapse of $\Delta\Psi$m, swelling of mitochondria, and MPP opening in the SHSY5Y cells is associated with activation of antiapoptotic Bcl-2 family proteins Bcl-2 and Bcl-xL and downregulation of Bad and Bax (Akao et al., 2002a,b; Weinreb et al., 2004). Evidence for this has come from the studies demonstrating that rasagiline increases Bcl-2 and Bcl-xL mRNA and protein (Fig. 1) but decreases Bax and Bad mRNA and protein in SHSY5Y and PC12 cells. In this regard, Bcl-2-overexpressing SHSY5Y cells behave identical to those treated by rasagiline in that they are resistant to neurotoxicity initiated by *N*-methy-*R*-salsolinol, SIN-1 and 6-hydroxydopamine. Inhibition of ubiquitin-proteasome system by *N*-methy-*R*-salsolinol, which re-

sults in cryptozoic translocation of GAPDH and activation of cytochrome c oxidase by the cytochrome c release, is also prevented by pretreatment with rasagiline. A similar result is also obtained with SHSY5Y cells overexpressing Bcl-2. Further support for Bcl-2 family protein participation in the neuroprotective activity of rasagiline has come from cDNA microarray gene expression and proteomic profiling in mouse midbrain resulting from chronic rasagiline treatment, where cell survival- (Bcl-2 and Bcl-xL) and death (Bax and Bad)-inducing family proteins are up- and downregulated, respectively (Sagi et al., 2003).

Structure-activity relationship studies with rasagiline and ladostigil and their non-MAO inhibitor *S* isomer derivatives (TVP1022 and TV3279) have indicated that the propargyl moiety in these drugs is crucial for the neuroprotective activity (Maruyama et al., 2003; Yogev-Falach et al., 2003). Their aminoindan metabolite is not antiapoptotic, however, and only in certain conditions does it have neuroprotective activity, as in the case of serum withdrawal in partially neuronally differentiated PC12 cells (Maruyama et al., 2003; Bar-Am et al., 2004a). The mechanism of neuroprotective action of rasagiline and its derivatives in some respects resembles that of cyclosporine A, which has been shown to be cardioprotective in heart myocytes and neuroprotective against a variety of neurotoxins, including *N*-methyl-*R*-salsolinol, SIN-1, and MPTP. Unlike cyclosporine A, however, rasagiline is unable to fully suppress the Ca^{2+}-induced MPP opening, as is seen with Bcl-2 overexpression and the action of bonkrekic A, which target respective components of the MPTp complex (Akao et al., 2002a,b). Nevertheless, in many respects the neuroprotective response of SHSY5Y cells to rasagiline is very similar to that of cells overexpressing Bcl-2 (Maruyama et al., 2001a, Akao et al., 2002). Such cells are also resistant to apoptosis by neurotoxins such as *N*-methyl-*R*-salsolinol, in which there is no collapse of $\Delta\Psi$m, inhibition of ubiquitin-proteasome system, release of cytochrome c, or GAPDH translocation from cytoplasm to the nucleus in response to the neurotoxin (Maruyama et al., 2001a; Akao et al., 2002a,b). Our present studies are directed at identifying upstream target protein(s) and at which VDAC protein site rasagiline acts upon in the mitochondria (Maruyama et al., 2001a,b).

LADOSTIGIL, A NOVEL ANTI-ALZHEIMER BIFUNCTIONAL CHOLINESTERASE-MAO INHIBITOR DERIVATIVE OF RASAGILINE

We have synthesized recently a series of novel propargylamine bifunctional drugs with a carbamate cholinesterase (ChE) inhibitory moiety in the pharmacophore of rasagiline to preserve its neuroprotective activity and inhibit MAO and acetyl ChE to increase dopaminergic and cholinergic transmissions (Weinstock et al., 2001). The *R* enantiomer of these compounds, ladostigil (TV3326, [(*N*-propargyl-[3*R*]aminoindan-5-yl)-ethyl methyl carbamate]), inhibits buturylesterase and ChE for a longer time than rivastigmine does, with a greater affinity for the former and a selective inhibitor for brain MAO-AB. It improves memory impairment in scopolamine-treated rats. Its *S* isomer, TV3279, is also a ChE inhibitor but has no MAO inhibitory activity, and has similar action in the scopolamine impairment test (Weinstock et al., 2000, 2001). As an MAO-AB inhibitor, it has anti-Parkinson activity in the MPTP mouse model (Sagi et al., 2003) and antidepressant activity in the forced swim test, because it raises brain levels of dopamine, serotonin, and noradrenaline (Weinstock et al., 2002, 2003; Sagi et al., 2003). These compounds retain the neuroprotective activities of rasagiline in response to various neurotoxins in partially differentiated PC12 and SHSY5Y neuroblastoma cells deprived of serum and NGF and in vivo (Weinstock et al., 2000, 2003; Youdim et al., 2001b). TV3326 (ladostigil) and TV3279, which possess the propargylamine moiety of rasagiline, share the same neuroprotective property and mechanism of action of the parent drug (Maruyama et al., 2003).

RASAGILINE, PKC, AND NEUROPROTECTION

PC12 and SHSY5Y neuroblastoma cell viability is reduced markedly by 24-hr serum withdrawal (75.8 $\pm$ 6% and 73.2 $\pm$ 7% of full-serum control, respectively). Rasagiline significantly prevents cell death induced by serum deprivation in PC12 and SHSY5Y neuroblastoma cells. Consistent with its antiapoptotic activity, rasagiline is able to prevent the appearance of the cleaved activated form of caspase-3 and the cleavage of the caspase substrate, PARP-1. This is PKC dependent, because the specific broad-spectrum PKC inhibitor GF109203X, which exhibits high affinity for conventional PKCs (α, β, and γ) as well as the novel isoenzyme PKCϵ (Ku et al., 1997; Gekeler et al., 1996) prevents this and the neuroprotective activity. Moreover, rasagiline decreases serum free-induced cleavage and activation of caspase-3 and PARP-1 and the increase in Bad and Bax that occurs in serum-free PC12 and SHSY5Y cells. These effects are prevented by the PKC inhibitor GF109203X and the MEK inhibitor PD98056 ET. These studies indicate the involvement of the PKC-MAP kinase-dependent pathway in rasagiline-stimulated cell viability and survival. The activation of PKC is associated with protection of neuronal cells (Durkin et al., 1997; Maher, 2001). Short-term treatment of PC12 cells with rasagiline dose-dependently induces significant PKC phosphorylation, which is inhibited by GF109203X (Weinreb et al., 2004). PKC translocation to the membrane fraction upon activation and membrane localization is used often as a marker for PKC activation. PMA is known to markedly induce translocation of p-PKC (pan), PKCα, and PKCϵ in PC12 cells (Wooten et al., 1994). Rasagiline has a similar activating effect on p-PKC, PKCα, and PKCϵ translocation to the membrane fractions. These results are supported by previous studies in which we have shown that rasagiline treatment activates PKC and its isoforms in rat and mouse hippocampus (Bar-Am et al., 2004a) and causes its translocation (Weinreb et al., 2004).

N-PROPARGYLAMINE AS THE NEUROPROTECTIVE MOIETY OF RASAGILINE AND LADOSTIGIL

Although structural activity studies with propargylamines have shown that the propargyl moiety may be responsible for the neuroprotective activity, *N*-propargylamine itself has been studied only recently (Weinreb et al., 2004). We have shown recently that *N*-propargylamine at low concentrations (1 and 10 μM) significantly reduces cell death induced by serum deprivation in PC12 and SHSY5Y neuroblastoma cells, with a concomitant decrease in activated caspase-3 and PARP. Moreover, treatment of PC12 cells with increasing concentrations of *N*-propargylamine results in a significant, dose-dependent increase in PKC phosphorylation; pretreatment with GF109203X and PD blocks the effect of propargylamine on PKC phosphorylation. Real-time RT-PCR analysis revealed that 24-hr treatment of PC12 cells with *N*-propargylamine significantly increased Bcl-xL and Bcl-w mRNA expression and decreased Bad and Bax mRNA expression, compared to the levels observed in serum-free cultures (Weinreb et al., 2004). In addition, similar to rasagiline and ladostigil, *N*-propargylamine increased PKCα and PKCε mRNA levels and reduced PKCγ mRNA levels compared to those detected in serum-free culture (Bar-Am et al., 2004a,b; Weinreb et al., 2004).

GENOMIC AND PROTEOMIC PROFILING OF RASAGILINE AND *N*-PROPARGYLAMINE NEUROPROTECTIVE ACTIVITY

To determine further the mechanism of rasagiline and *N*-propargylamine-neuroprotection, we carried out in cultured PC12 cells customized cDNA microarray gene expression changes in cell-survival and death-related genes, including selected Bcl-2 and PKC family members (Weinreb et al., 2004). Real-time RT-PCR consisted of RNA samples isolated from PC12 cells, maintained in full-serum or serum-free media and treated with or without rasagiline (0.01–10 μM) for 24 hr. Expression of each gene was normalized to the housekeeping gene 18S rRNA, because this transcript is reportedly less susceptible to influence by external factors (Schmitten and Zakrajsek, 2000). Real-time RT-PCR demonstrated that in serum-free culture, Bcl-2, Bcl-w, and Bcl-xL expression levels were reduced significantly (~60% of that in full-serum culture), whereas Bad and Bax mRNA levels were increased markedly (~1.8- and ~2.5-fold, respectively, vs. full-serum culture). Rasagiline treatment for 24 hr significantly induced Bcl-xL and Bcl-w mRNA expression (at 1 μM, by ~2- and ~1.35-fold, respectively, vs. serum-free culture). In addition, rasagiline markedly reduced mRNA of Bad and Bax expression (at 1 μM, ~70% of that in serum-free culture).

Examination of the effect of rasagiline on PKC gene expression by real-time RT-PCR analysis using RT primers specific for PKCα, PKCε, PKCδ and PKCγ revealed that in serum-free PC12 culture, PKCα and PKCε mRNA expression levels are reduced significantly whereas PKCδ and PKCγ mRNA levels were increased markedly. Treatment of serum-free PC12 cells with rasagiline (1 and 10 μM) for 24 hr upregulated PKCα and PKCε mRNA levels, compared to the decreased expression detected in serum-free culture. In addition, rasagiline downregulated the increased PKCγ mRNA level observed in serum-deprived cells. Moreover, quantitative real-time RT-PCR pointed to an association between the mechanism of rasagiline neuroprotective action and brain-derived neurotrophic factor (BDNF) gene expression. BDNF gene expression downregulation occurring in serum-deprived PC12 cells are reversed by treatment with 1 and 10 μM of rasagiline with a 3.5-fold increase in BDNF (Weinreb et al., 2004). The ability of rasagiline to increase BDNF mRNA (Weinreb et al., 2004) and induce the release of glia cell line-derived neurotrophic factor (GDNF) in SHSY5Y cells (Maruyama et al., 2004) may point to its neurorescue via activation of neurotrophic cell surface receptors. Almost identical results have been obtained with *N*-propargylamine (Weinreb et al., 2004).

DISCUSSION

Our studies have demonstrated clearly that the neuroprotective activity of rasagiline and its derivatives is associated with the propargyl moiety in these drugs. These compounds activate the Blc-2 antiapoptotic family proteins Bcl-2 and Bcl-xL and downregulate proapoptotic Bad and Bax. Given that the antiapoptotic activity of rasagiline and *N*-propargylamine in serum-free cells is blocked by PKC and MEK inhibitors clearly supports a role for PKC and MEK involvement in their neuroprotective mechanisms. (Fig. 1). These findings are complementary in activation of PKCα and PKCε in serum-deprived PC12 cells, the isoforms essentially involved in cell survival pathways. Furthermore, real-time PCR analyses have revealed that exposure of serum-deprived PC12 cells to rasagiline and *N*-propargylamine markedly increases PKCα and PKCε gene expression but decreases elevated PKCγ mRNA levels. Previous studies showed that that PKCγ was increased in ischemia (Selvatici et al., 2003), in which rasagiline is protective (Speiser et al., 1999) and decreased by the immunosuppressant cerebroprotective agent FK506 (Katsura et al., 2003).

Certain PKC isoforms are thought to act to deliver survival signals that protect against cell death. For instance, PKCα was shown to phosphorylate Bcl-2 at a site that increases its antiapoptotic function (Ruvolo et al., 1998), whereas overexpression of PKCε results in increased expression of Bcl-2. Suppression of PKCα triggers apoptosis through downregulation of Bcl-xL (Hsieh et al., 2003). In addition, MAPK/ERK cascades, which have been shown to inhibit cell death in a number of systems, can be activated by PKC. PKCα thus phosphorylates and activates Raf-1, an upstream kinase in the MAPK/ERK pathway (Kribben et al., 1993). Indeed our proteomic analysis has shown clearly that in vivo rasagiline upregulates Ras, Raf-1, PI3K, and AKT (Sagi et al., 2003). PKCε regulates

ERK-1 and -2 activation; and pharmacologic inhibition of MAPK/ERK signaling blocks phorbol ester-induced protection of neuronal cells against glutamate toxicity (Maher, 2001). These findings explain the activation of the MAPK/ERK cascade by rasagiline, ladostigil, and *N*-propagrylamine (Yogev-Falach et al., 2002, 2003). The involvement of PKC pathway in rasagiline and *N*-propargylamine-induced inactivation of the proapoptotic Bcl-2 family members, Bad and Bax, is consistent with PKC-dependent pathway promoting cell survival via phosphorylation and inactivation of Bad-mediated cell death (Tan et al., 1999).

Proteomic analysis of midbrain of mice treated in vivo with rasagiline alone or its prevention of parkinsonian syndrome in MPTP-induced nigrostriatal dopamine neuron death have demonstrated that this drug has complex mechanism of neuroprotective activity that involves several cascades. These include activation of PKC-MAP kinase pathways identified with cultured PC12 and SHSY5Y cells (Weinreb et al., 2004; Bar-Am et al., 2004b), the neurotrophic factors GDNF, BDNF, and NGF and the PI3K-AKT pathway via upregulation of Ras (Sagi et al., 2003; Maruyama et al., 2004; Weinreb et al., 2004) and downregulation of Fas and Fas ligand, which interact with Bad and Bax on the outer mitochondrial membrane (Sagi et al., 2003).

Rasagiline increases the expression of BDNF, GDNF, and NGF (Fig. 1), neurotrophins found to promote survival of all major neuronal types affected in AD and PD animal models (Murer et al., 2001). Reduced BDNF expression was demonstrated in the substantia nigra of individuals with PD (Parain et al., 1999) and BDNF prevented spontaneous death of dopaminergic neurons in rat primary mesencephalic culture (Hyman et al., 1991) and the reduction in striatal dopamine content induced by MPTP in mice (Huang et al., 1999). Interestingly, BDNF was also reported to regulate PKC activation (Tremblay et al., 1999) and affect Bcl-w and Bcl-xL expression (Middleton et al., 2001). Moreover, rasagiline was reported recently in SHSY5Y cells to increase expression and release of GDNF (Maruyama et al., 2004), another neurotrophic factor that may have specificity for dopamine neurons. Recent clinical studies with a central infusion of this factor in PD subjects have shown partial recovery from PD. The ability of rasagiline to activate antioxidant enzymes superoxide dismutase (SOD) and catalase (Carrillo et al., 2000) suggests that these, together with its action on Bcl-2 family protein and PKCs, can suppress the death process and promote survival of dopamine neurons (Fig. 1) (Maruyama et al., 2004). These results may explain those of recent controlled studies with rasagiline in PD in which the decline in disability could not be explained by the symptomatic effect of the drug but may have been due to a disease-modifying activity of rasagiline (Parkinson Study Group, 2004) Further study will clarify the interrelationship between rasagiline-induced BDNF, GDNF, or other neurotrophic substances, PKC signaling pathway, Bcl-2-related protein family, and neuroprotection in our animal studies and its possible disease modifying activity not only in PD subjects but also in AD.

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