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NEUROPHARMACOLOGICAL, NEUROPROTECTIVE AND AMYLOID PRECURSOR PROCESSING PROPERTIES OF SELECTIVE MAO-B INHIBITOR ANTIPARKINSONIAN DRUG, RASAGILINE

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Summary

Rasagiline (*N*-propargyl-1*R*-aminoindan) is a novel, highly potent, irreversible monoamine oxidase (MAO)-B inhibitor designed for use as an antiparkinsonian drug. Unlike selegiline, rasagiline is not derived from amphetamine or metabolized to neurotoxic l-methamphetamine derivative, and it does not have sympathomimetic activity. Moreover, at selective MAO-B inhibitory dosage, it does not induce a "cheese reaction." Rasagiline is effective as monotherapy or as an adjunct to L-dopa for patients with early and late Parkinson's disease. Adverse events do not occur with greater frequency in subjects receiving rasagiline than in those on placebo. Its *S*-isomer, TVP1022, is more than a thousand times less potent as an MAO inhibitor. However, both drugs have neuroprotective activities in neuronal cell cultures in response to various neurotoxins, as well as *in vivo* (*e.g.*, in response to

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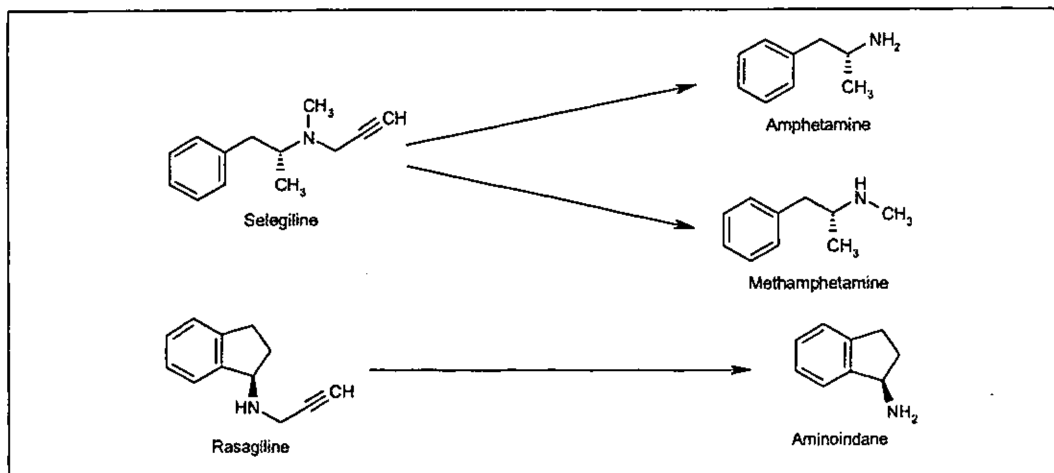

Fig. 1. The structures of rasagiline, selegiline and their respective metabolites aminoindan and l-methamphetamine.

global ischemia, neurotrauma, head injury, anoxia, etc.), indicating that MAO inhibition is not a prerequisite for neuroprotection. The neuroprotective activity of these drugs has been demonstrated to be associated with the propargylamine moiety, which protects mitochondrial viability and mitochondrial permeability transition pore by activating Bcl-2 and downregulating the Bax family of proteins. Rasagiline and its newly developed cholinesterase inhibitor derivatives process amyloid precursor protein (APP) into the neuroprotective-neurotrophic soluble APPalpha (sAPPα) by protein kinase C- and mitogen-activated protein kinase-dependent activation of α-secretase, and they increase nerve growth factor, GDNF and BDNF expression and proteins. Thus, rasagiline and its derivatives may induce neuroprotection, neuroplasticity and long-term potentiation. Rasagiline has therefore been chosen by the National Institutes of Health (NIH) to study its neuroprotective effects in neurodegenerative diseases. Long-term studies are required to evaluate the drug's disease-modifying prospects in Parkinson's and Alzheimer's diseases. © 2005 Prous Science. All rights reserved.

Introduction

The knowledge that dopamine is oxidatively deaminated equally well by monoamine oxidase (MAO) types A and B (1), the dominance of MAO-B (80%) as compared to MAO-A in the extrapyramidal regions of human brain (1–3) and the absence of the "cheese reaction" in whole animal and isolated tissue preparations by the selective irreversible MAO-B inhibitor selegiline (deprenyl) (4) led us to introduction of this irreversible inhibitor as an adjunct to levodopa therapy for Parkinson's disease (5–7). Selegiline has been a useful antiparkinsonian drug, both in monotherapy (8) and as an adjunct to levodopa therapy. Additionally, it has levodopa-sparing action (6, 9, 10). Selegiline is a propargyl derivative of l-methamphetamine. It irreversibly inhibits MAO-B by binding mole per mole covalently to the N5 position of the isoalloxazine moiety of FAD, the cofactor of MAO-B (11, 12). *In vivo*, selegiline is metabolized to its major metabolites l-amphetamine and l-methamphetamine (13) (Fig. 1). In behavioral (14), pharmacological preparations and *in vivo*, it possesses amphetamine-like and sympathomimetic actions (15, 16), which result in its ability to increase blood pressure and heart rate. Its major metabolite, l-methamphetamine, has neurotoxic action. However, as a consequence of not having similar structural drugs, it has not been possible to evaluate selegiline's antiparkinsonian activity or its mechanism of action.

We have identified and developed the highly potent irreversible selective inhibitor of MAO-B, rasagiline [*N*-propargyl-1*R*(+)-aminoindan; TVP-1012] (Fig. 1) (17–22). Rasagiline has been developed as an antiparkinsonian drug (17, 23–27). It is a propargylamine derivative of aminoindan, and unlike selegiline, it is not metabolized to amphetamine but to aminoindan, which has no amphetamine-like sympathomimetic properties (Fig. 1). Rasagiline is an *R*-isomer of the racemic form of the selective

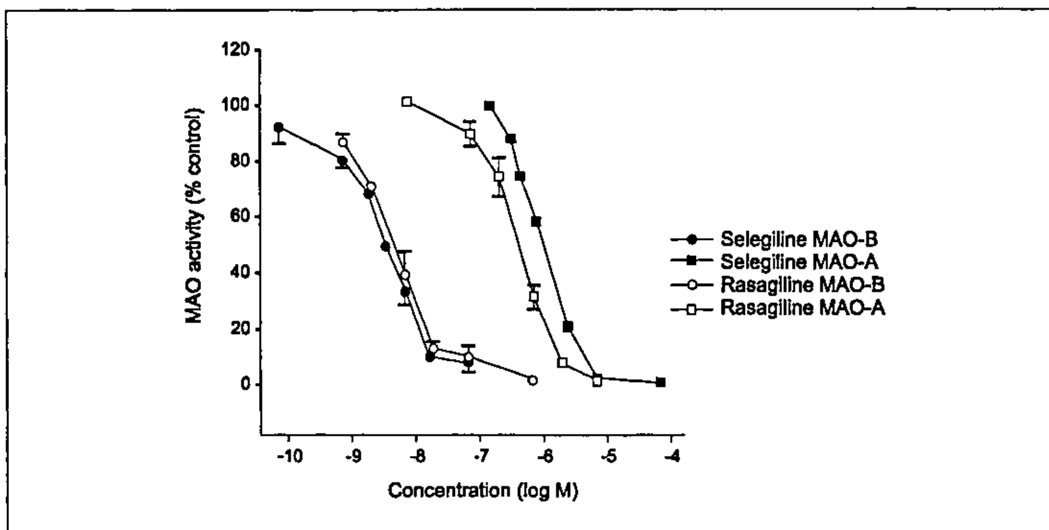

Fig. 2. Comparative *in vitro* MAO inhibitory activities of rasagiline and selegiline in rat brain mitochondrial preparations. Similar but lower effects were seen with liver MAO inhibition (ref. 22).

MAO-B inhibitor, AGN1135 (20, 21). Similarly to selegiline at its selective MAO-B inhibitory dosage, it does not produce the "cheese reaction" in isolated tissue preparations or *in vivo* in rats and cats (16, 28).

Several controlled phase III studies have demonstrated that rasagiline is effective as a monotherapy drug in early and late Parkinson's disease, and as an adjuvant to L-dopa, it significantly reduces the off effect (26, 27, 29, 30, 31).

Monoamine oxidase inhibitory activity of rasagiline

Rasagiline's MAO-A and -B inhibitory activities have been determined *in vitro*, as well as acutely and chronically *in vivo* in various tissues of mice, rats, cats and monkeys, together with the activities of its S-isomer TVP1022 and of the racemic compound AGN-1135, in comparison with the activity of selegiline (l-deprenyl) (Fig. 2). The tissues that were studied for MAO inhibition included brain, liver and small intestine. Rasagiline and its racemic form are highly potent, selective, irreversible inhibitors of MAO *in vitro*, with similar potency to that of selegiline (Fig. 2). However, the S-isomer (TVP-1022) is relatively inactive *in vitro* and *in vivo* in the tissues studied. The *in vitro* IC_{50} values for inhibition of rat brain MAO activity by rasagiline are 4.43 ± 0.92 nM (type B) and 412 ± 1.23 nM (type A). The ED_{50} values for *ex vivo* inhibition of MAO-B in the brain and liver by a single dose of rasagiline are 0.1 ± 0.01 and 0.042 ± 0.0045 mg/kg, respectively. These same values for MAO-A inhibition are 8.35 ± 2.2 and 2.42 ± 0.39 mg/kg, respectively. *In vivo* rasagiline is between 10–20 times more potent than selegiline, and it maintains its selective MAO-B inhibition in the liver and brain when administered as chronic (21-day) oral dosage, with ED_{50} values of 0.014 ± 0.002 and 0.013 ± 0.001 mg/kg, respectively, for these tissues. The degree of selectivity of rasagiline for inhibition of MAO-B as opposed to MAO-A *in vitro* is similar to that of selegiline, with rasagiline being significantly more potent *in vivo*. In fact, rasagiline produces near complete inhibition of MAO-A and -B at doses of 10 and 0.4 mg/kg, respectively (22). This may explain its greater potency for inhibition of human platelet MAO-B. In addition, a rasagiline dose as low as 0.5–2 mg/day has shown efficacy in parkinsonian subjects (27).

Mechanism of monoamine oxidase B inhibition by rasagiline

The inhibitory potencies of rasagiline and its *S*-isomer have been examined in brain, liver and intestine of rats after acute and chronic oral treatment (22) and compared to that of the selective irreversible MAO-B inhibitor, selegiline (4). The results demonstrated a high degree of stereo selectivity

for MAO inhibition by rasagiline and its *S*-isomer. This is reflected in the fact that TVP1022 (the *S*-isomer of rasagiline) is three orders of magnitude less potent as a MAO inhibitor *in vitro*. In contrast, selegiline and its isomer show only a small degree of stereo selectivity, and in this case the *S*(–) enantiomer is more potent than the *R*(+) enantiomer, opposite to that of rasagiline. The full explanation for this markedly different degree of stereo selectivity between closely allied chemical compounds has become apparent with three-dimensional modeling of the MAO active site. Indeed, the recent crystallographic studies with MAO-B, rasagiline and TVP-1022 by Binda *et al.* (32) and Edmondson *et al.* (33) have clearly shown that while rasagiline enters the MAO-B active site gorge and binds to it irreversibly, TVP1022 makes a 180-degree turn to enter the gorge (unpublished data) and slowly but eventually inhibits the enzyme.

Rasagiline, similar to selegiline, is a propargyl-containing drug. However, the propargyl component in rasagiline, unlike that of selegiline, is bound to an aminoindan moiety. The propargyl-containing MAO inhibitors such as clorgyline, pargyline and selegiline are known as suicide inhibitors since they are substrates of the enzyme (11, 34, 35). They inactivate MAO-A and -B selectively and irreversibly by time- and concentration-dependent mechanisms by interacting covalently with the cysteinyl-FAD cofactor of the enzymes at their active centers (36, 37). This interaction takes place between the propargyl group and the N5 of the FAD isoalloxazine component of liver (11) and brain MAO (38). It has been shown for these inhibitors that one mole of the inhibitor inactivates one mole of the enzyme (34), and since rasagiline has the same enzyme-inactivating moiety as those of the aforementioned propargylamines, its mechanism of MAO inactivation is considered to be identical to that of these inhibitors. The 1:1 interaction of propargylamine MAO inhibitors with MAO-A and -B has been used to determine the molecular turnover of these enzymes in the estrus cycle and their response to sex steroids (39), as well as their regulation with steroids (40, 41). The estrus cycle has a profound effect on MAO-A activity, with progesterone inducing and estradiol reducing MAO-A enzyme activity. Studies with endothelial and chromaffin cell cultures, which contain, respectively, MAO-A and -B only, have shown that these steroids affect only synthesis of active MAO-A and its turnover, and not those of MAO-B (41). No adequate explanation has been put forward as to why steroids do not alter MAO-B expression, what regulates this enzyme, what its function is in the brain and whether this enzyme has other endogenous substrates, such as *N*-acetyl derivatives of diamine oxidase substrates (*e.g.*, spemine spemidine, puetrecine) (42), besides dopamine and phenylethylamine.

A comparison of turnover of rat striatal MAO-B inhibition by rasagiline and selegiline shows remarkable similarities between the two compounds (43). Striatal MAO-B has a significantly slower turnover as compared to MAO-A. It is apparent that rasagiline is a very potent selective MAO-B inhibitor and has a good uptake across the blood-brain barrier, as shown by the similarity of inhibition curves between liver and brain (44). Although when compared *in vitro*, rasagiline had similar potency to selegiline for inhibition of MAO-B, the *in vivo* study showed a greater potency for rasagiline. This greater potency of rasagiline is even more marked if, instead of 50% enzyme inhibition, the dose required for 80% inhibition is measured. The reason for this is not currently known, but it may be due to different rates of metabolism of the parent compounds *in vivo* or to improved tissue penetration of rasagiline. Studies in humans show an approximately 5- to 10-fold greater potency for rasagiline over selegiline for inhibition of platelet MAO-B (unpublished data). Although rasagiline has a greater potency than selegiline, its selectivity for MAO-A and -B inhibition is very similar to what has been reported for selegiline.

The results from studies on rat tissues complement findings in nonhuman primate (monkey) brains (45), where rasagiline was given chronically for 7 days at various doses and MAO-A and -B activities were measured in several brain regions, including caudate nucleus, globus pallidus, cerebral cortex and hippocampus. Rasagiline was shown to be a potent selective inhibitor of MAO-B in the caudate nucleus and globus pallidus, where the activity of MAO-B, similarly to in the human brain, is 4-fold higher than that of MAO-A (45). In human volunteers and parkinsonian subjects, a 0.5, 1.0 or 2.0 mg daily dosage given chronically is sufficient to completely inhibit platelet MAO, which is solely type B. This potency will most likely be reflected in the human brain, since Riederer and Youdim (46) showed that a 10 mg daily dose of selegiline given to parkinsonian subjects fully inhibited platelet MAO-B with a similar inhibitory potency in caudate nucleus, substantia nigra, globus pallidus and thalamus,

in contrast to a limited MAO-A inhibition in these brain regions, as determined at autopsy from subjects on chronic selegiline. Unlike the studies in rat brain, where MAO-A or -B inhibitors do not affect dopamine levels (47), in human brain there was a significant, but moderate, increase (45%) in dopamine levels (enriched by MAO-B), as well as a highly significant increase (3,000%) in the MAO-B substrate phenylethylamine in these regions (48). In contrast, the limited MAO-A inhibition was observed in these regions, and there were no appreciable effects on serotonin or noradrenaline and their metabolites in these regions. Thus, selective inhibition of brain MAO-B can be maintained with the right dosages of selegiline, and it would not be expected to be different for rasagiline, as discussed above regarding studies on rat brain *in vivo* (22).

MAO-A and -B activity recovery after *in vivo* inhibition by rasagiline, which is related to the synthesis of enzyme apoprotein, differs significantly between tissues such as liver, small intestine and brain. The small intestine MAO-B activity has the fastest recovery, while the brain MAO-B activity shows the slowest recovery. In comparison, MAO-A activity recovery in rat brain is significantly faster and the half-life is 3–5 days. These differences in rat brain enzyme activity recovery after rasagiline treatment are not unusual: similar findings have been reported for enzyme recovery after inhibition by selegiline and clorgyline (49, 50). Indeed, a recent comparative study of rasagiline and selegiline showed almost identical results, with a half-life in the striatum of 7–9 days. However, the half-life for recovery of MAO-B after selegiline treatment has been reported to be well over 30 days (40) in primate (monkey and human) brains and 13 days in the rat brain (49, 50).

Neuropharmacology of rasagiline

The effects of rasagiline on CNS monoamine levels, behavioral response to fluoxetine, reserpine reversal and cardiovascular responses to tyramine have been studied in a range of doses in rats (51). Rasagiline reverses reserpine-induced ptosis at doses above 2 mg/kg, which are nonselective for MAO-B, but not at MAO-B-selective doses. Combination of rasagiline, at doses up to 20 mg/kg, which is well above its selectivity for MAO-B, with fluoxetine (10 mg/kg) does not induce the behavioral serotonergic syndrome seen following nonselective MAO-A + -B inhibition by traylcypromine, and unlike the latter inhibitor, it does not induce behavioral serotonergic nor dopaminergic syndrome in response to l-tryptophan or L-dopa, respectively (51). Similarly to selegiline (14, 47), when administered orally it does not alter hippocampal or striatal levels of noradrenaline, 5-hydroxytryptamine (5-HT) or dopamine after single or chronic doses of rasagiline up to 2 mg/kg. At doses higher than this, it causes an increase in hippocampal 5-HT and a reduction in 5-hydroxyindole acetic acid (5-HIAA). Striatal levels of dihydroxyphenylacetic acid (DOPAC) are reduced at doses above 1 mg/kg, suggesting inhibition of both MAO-A and -B. However, with chronic oral administration of doses up to 1 mg/kg daily for 21 days, levels of noradrenaline, 5-HT and dopamine in hippocampus and striatum, respectively, are unaffected, but 5-HT increases at 2 mg/kg and striatal dopamine content increases at 2 and 5 mg/kg. It is most likely that at these doses MAO-A is also inhibited, leading to the increase in these neurotransmitters, which are metabolized by MAO-A (14, 47). Rasagiline, similarly to selegiline and reversible MAO-B inhibitors (*e.g.*, lazabemide and milacemide), does not potentiate pressor responses to oral tyramine when administered as single oral doses of up to 5 mg/kg. However, doses of 10 mg/kg do significantly potentiate this response. At this dose, both MAO-A and -B are inhibited, and it is the irreversible inhibition of MAO-A that induces the cheese reaction (16, 52). Similarly, pressor responses to tyramine (5, 10 and 20 mg/kg) are not significantly potentiated following 21-day chronic treatment with rasagiline up to 2 mg/kg daily. Thus, rasagiline does not modify CNS monoamine tissue levels or pressor responsiveness to tyramine at doses selective for inhibition of MAO-B. The greater potency of rasagiline in comparison to selegiline is also reflected in humans, since a 1–2 mg daily dose of rasagiline inhibits platelet MAO-B. Parkinsonian subjects respond to rasagiline doses as low as 0.5–2 mg daily administered as controlled monotherapy and as an adjunct to L-dopa therapy in early and late Parkinson's disease studies (26, 27, 53). This value is significantly lower than the 10 mg daily dosage employed with selegiline. These results, together with the lack of tyramine sympathomimetic potentiation by rasagiline at selective MAO-B inhibitory dosage, indicate that this inhibitor may be a more efficient drug than selegiline in the treatment of Parkinson's disease as either symptomatic or levodopa adjunct therapy. Furthermore, its lack of amphetamine-like metabolites could confer a therapeutic advantage to rasagiline.

In vivo dopamine release and turnover

Acute inhibition of MAO-B with selegiline or rasagiline in rats does not alter striatal dopamine metabolism (47), but both drugs chronically increase *in vitro* and *in vivo* release of dopamine (54). The incremental release of striatal dopamine induced by depolarizing concentrations of K^+ is significantly greater with chronic clorgyline (a MAO-A inhibitor), selegiline and rasagiline treatments, where MAO-A and -B are selectivity inhibited (55). Single doses of selegiline and rasagiline have no significant effects on striatal dopamine efflux (56). In these studies, only clorgyline reduced dopamine metabolism, whereas dopamine release was enhanced by both chronic MAO-A and -B inhibitor administration. The possibility that an inhibition of high-affinity dopamine transporter by selegiline and rasagiline may be the resultant effect has also been investigated (57). Striatal levels of dopamine transporter are elevated with acute and chronic selegiline administration, but not with rasagiline, clorgyline, nomifensine or amphetamine. Thus, an increase rather than a decrease in dopamine transporter expression appears to be a special property of selegiline and not of other inhibitors. Selegiline is a derivative of l-amphetamine and its major metabolite is l-methamphetamine. It has been suggested that dopamine release may result from this metabolite. However, since rasagiline is not metabolized to amphetamine and has a similar action on striatal dopamine release, this effect is not dependent on the production of amphetamine. The most likely explanation for this effect is the metabolic inhibition of phenylethylamine, a selective substrate of MAO-B, which is highly elevated in response to selegiline in rat brain (58) and parkinsonian brain obtained at autopsy (48). In contrast, neither dopamine, serotonin or noradrenaline metabolism are altered by rasagiline or selegiline in rat brains (14, 47, 59, 60). Although selegiline has been shown to cause a modest but significant increase in parkinsonian striatal dopamine (46), it also profoundly affects phenylethylamine metabolism, with a more than 3,000% increase in parkinsonian striatum levels obtained at autopsy in patients on l-deprenyl therapy for between 4 weeks and 4 years (48). As a specific substrate of MAO-B (58, 61), phenylethylamine is increased in rat brains treated with selegiline. Phenylethylamine is a highly potent releaser of dopamine, as well as an inhibitor of neuronal uptake and a competitive inhibitor of MAO-B. Indeed, at their selective *in vivo* MAO-B inhibitory doses, both selegiline and rasagiline potentiate the behavioral response to exogenous treatment with phenylethylamine (43). This response is only observed when MAO-B is inhibited by more than 80% (62, 63). These results may have significant implications for understanding the therapeutic action of rasagiline when administered as monotherapy in early and late Parkinson's disease, or when used as an adjuvant to L-dopa. The continuous release of dopamine after chronic rasagiline and selegiline in rat brain (54), together with the long-term selective inhibition of MAO-B in extrapyramidal regions (*i.e.*, caudate nucleus, substantia nigra, globus pallidus and thalamus) (46), may explain why the initial clinical response to rasagiline monotherapy in parkinsonian subjects continues even 8 weeks after the termination of the drug (29), whose half-life is considered to be greater than 30 days (40). There could also be another explanation, such as adaptive postsynaptic dopamine receptor sensitization, but this has not so far been shown for either selegiline or rasagiline.

Neuroprotective activity of rasagiline

In cell culture and in vivo

Rasagiline possess neuroprotective activity against cell death, as demonstrated *in vitro* (in neuronal cell cultures) and in several *in vivo* preparations in response to neurotoxins and neurotoxic events and serum and nerve growth factor withdrawal in neuronal cell culture (Tables I and II). Rasagiline is among a handful of agents chosen for further study as potential neuroprotective compounds in clinical neuroprotection trials in Parkinson's disease (64). Clinically as an antiparkinsonian drug (26, 27) and experimentally as a MAO-B inhibitor (22, 51), rasagiline is 10–20 times more potent than other agents studied. Similarly, selegiline and akyl-propargylamines exhibit neuroprotective activities in several neuronal cell culture models, as well as *in vivo* in response to a number of neurotoxins (65–71). This property is not related to

Table I: In vivo *neuroprotective activity of rasagiline in models of neurodegenerative diseases.*

- MPTP model of Parkinson's disease (73)
- Globa ischemia (74, 75)
- Neurotruma model of head injury (76)
- Amyotrophic lateral sclerosis (77)
- Eae model of multiple sclerosis?
- Heart ischemia?
- 6-Hydroxydopamine? (78)

Table II. In vitro *neuroprotective activity of rasagiline in neuronal cell cultures.*

Neurotoxic agents	Cell type	
	PC-12	SHSY-5Y neuroblastoma
Glutamate	+	+
SIN-1	ND	+
N-methyl-(*R*)-salsolinol	ND	+
6-Hydroxydopamine	ND	+
Serum and nerve growth factor depravation	+	ND
Glucose and oxygen depravation	+	ND
Okdanoic acid+	+	
Aβ-amyloid aggregate	+	+
α-Synuclein-Fe aggregate	+	+
TNF-α		

the MAO-B inhibitory activity of either rasagiline and its novel cholinesterase-MAO inhibitor derivatives or selegiline (44, 72, 79–81), since the *S*-optical isomer of rasagiline, TVP1022, which has poor MAO-B inhibitory activity, exhibits similar neuroprotective activity (66, 67, 70, 71, 82). Clinical trials with selegiline (83) failed to establish its neuroprotective activity because its symptomatic effect could not be distinguished from a possible neuroprotective action. One reason why neuroprotection was not observed with selegiline in clinical trials (83) may be a consequence of l-methamphetamine generation, which could interfere with selegiline's neuroprotective activity (79, 80, 84–86). Although rasagiline (72) and selegiline have similar structures, selegiline's major metabolites l-amphetamine and l-methamphetamine are thought to be neurotoxic (84–86). However, rasagiline's metabolite is aminoindan, which has been reported to have neuroprotective activity in its own right (87, 88). Indeed, Abu-Raya *et al.* (85) and Bar Am *et al.* (86) have demonstrated that l-methamphetamine interferes with the neuroprotective action of selegiline and rasagiline in oxygen-glucose deprivation-induced cell death in nerve growth factor-differentiated PC-12 cells. These findings were confirmed with PC-12 cell cultures in the absence of serum and nerve growth factor. Furthermore, in these studies aminoindan, the major metabolite of rasagiline, prevented the neurotoxicity of l-methamphetamine, the major metabolite of selegiline (86), supporting the results from *in vivo* neuroprotective studies with aminoindan (87, 88) (Fig. 3).

Similarly to selegiline, rasagiline and TVP1022 protect against serum and nerve growth factor withdrawal-induced apoptotic death in partially differentiated PC-12 cells (and SHSY-5Y neuroblastoma cells, data not shown). Moreover, their activity appears to be superior to that of selegiline (Fig. 3). The results of these studies are very similar to the neuroprotective actions observed for rasagiline and selegiline in the prevention of PC-12 cell death during oxygen-glucose deprivation. Serum and nerve growth factor withdrawal in partially neuronally differentiated PC-12 cells results in decreased mRNA and protein of Bcl-2 and SOD, while those of Bax increase (22, 89). Rasagiline can substitute for serum and nerve growth factor to restore these proteins, an action that can be prevented with transcriptional and translational inhibitors cycloheximide and actinomysin D (Fig. 3). These findings suggest that rasagiline induces the gene-dependent synthesis and degradation of mitochondrial cell survival proteins. Furthermore, the neuroprotective activity of rasagiline, both *in vitro* in PC-12 and SHSY-5Y cells and *in vivo*, is associated with its induction of antiapoptotic SOD and catalase (90). The *in vivo* induction of Mn-zinc SOD and catalase by rasagiline was mainly associated with substantia nigra and kidney, two dopamine-rich tissues, without a substantial effect in the hippocampus (90). The induction of these enzymes was concentration and time dependent. The potent MAO-B inhibitory activity of rasagiline, which resides in the interaction of its propargylamine moiety with FAD co-factor of the enzyme (22), is unrelated to its neuroprotective activity (44, 66, 67, 70, 71). This view is supported by the observations that its *S*-optical isomer TVP1022 (44, 66, 71) and other derivatives (81), which have more than 1000-fold lower MAO-B inhibitory activities (22), have similar neuropro-

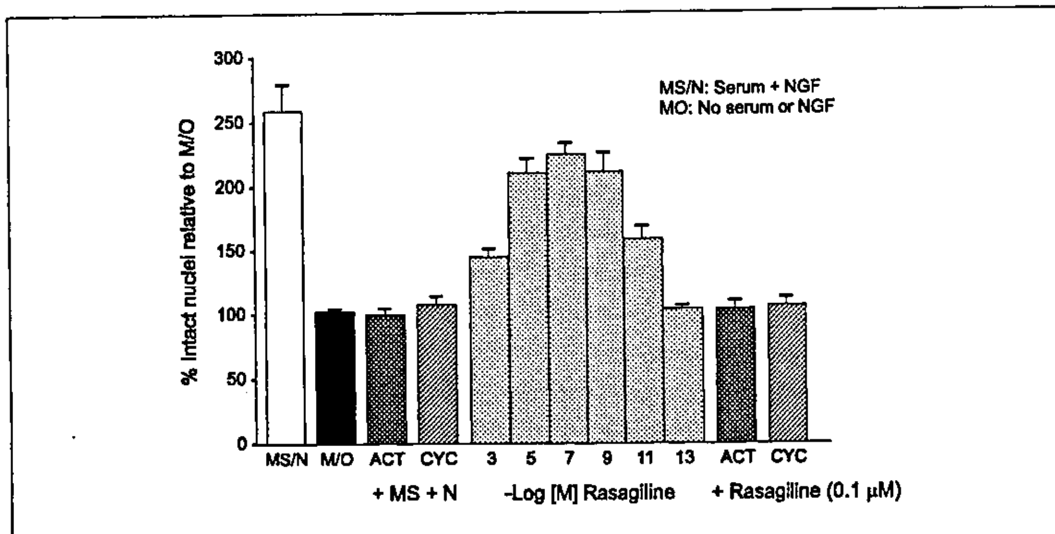

Fig. 3. The prevention of apoptotic death in serum- and nerve growth factor (NGF)-deprived PC-12 cells by rasagiline. The effects of transitional and transcriptional blocked by cycloheximide and actinomycin D (ref. 44).

tective activities in cell culture and *in vivo* (44, 66–69, 91). Furthermore, PC-12 cells and human SH-SY5Y neuroblastoma cells contain only MAO-A (92, 93), and the concentration of rasagiline employed in neuroprotective studies would not be sufficient to inhibit this enzyme. It is apparent that the neuroprotective activity of rasagiline and TVP1022 may be superior to that of selegiline, as reported by Abu-Raya *et al.* (85), Maruyama *et al.* (70, 71) and Bar Am *et al.* (86) (Fig. 4). This superior neuroprotective activity may result from the differences between the structure of selegiline and those of rasagiline and its derivatives. Selegiline is a derivative of amphetamine and is metabolized to methamphetamine *in vivo*, and similarly to amphetamine, it is a sympathomimetic compound (15, 25). Methamphetamine is known to be neurotoxic to dopaminergic neurons *in vivo* and to PC-12 and neuroblastoma cells *in vitro* (86, 94). Not only do the studies with l-methamphetamine confirm its neurotoxicity in cell culture, but we have also shown that unlike the aminoindan metabolite of rasagiline, l-methamphetamine prevents the neuroprotective activity of selegiline as well as that of rasagiline (Fig. 4). However, rasagiline is an aminoindan derivative, and neither it nor aminoindan is vasoactive (51). Unlike methamphetamine, aminoindan has little effect on sympathetic nervous function. Furthermore, both compounds demonstrate neuroprotective activity in serum- and nerve growth factor-free PC-12 cell cultures. Against l-methamphetamine, the major metabolite of selegiline (81, 86) (Fig. 4), it has been established that the neuroprotective activity of propargylamines such as rasagiline, its derivative and selegiline resides in the propargylamine moiety. Our recent study on the neuroprotective activity of rasagiline in PC-12 cells (86), together with previous observations (70, 71, 81, 85), indicate that rasagiline may have a greater neuroprotective potency. This may be attributed to a combination of the propargylamine moiety and the aminoindan backbone of the drug. Indeed 4-hydroxyaminoindan was reported to be protective in global ischemia in gerbils. Rasagiline prevents the neurotoxicity of glutamate in PC-12 cells (unpublished data) and primary cultures of rat hippocampal neurons (25), as well as in global ischemia in gerbils (87). The metabotropic glutamate 1 (mGlu1) receptor antagonist, 1-aminoindan-1,5-dicarboxylic acid (AIDA), which reduces neuronal death following oxygen-glucose deprivation in murine cortical cells and rat organotypic hippocampal cultures and after *in vivo* global ischemia in gerbils, possesses an aminoindan to which the dicarboxylic moiety is attached at its 1 position (95). The structural similarity in propargylamine attachment to position 1 of aminoindan of rasagiline and its pharmacological neuroprotective activity to AIDA cannot go unno-

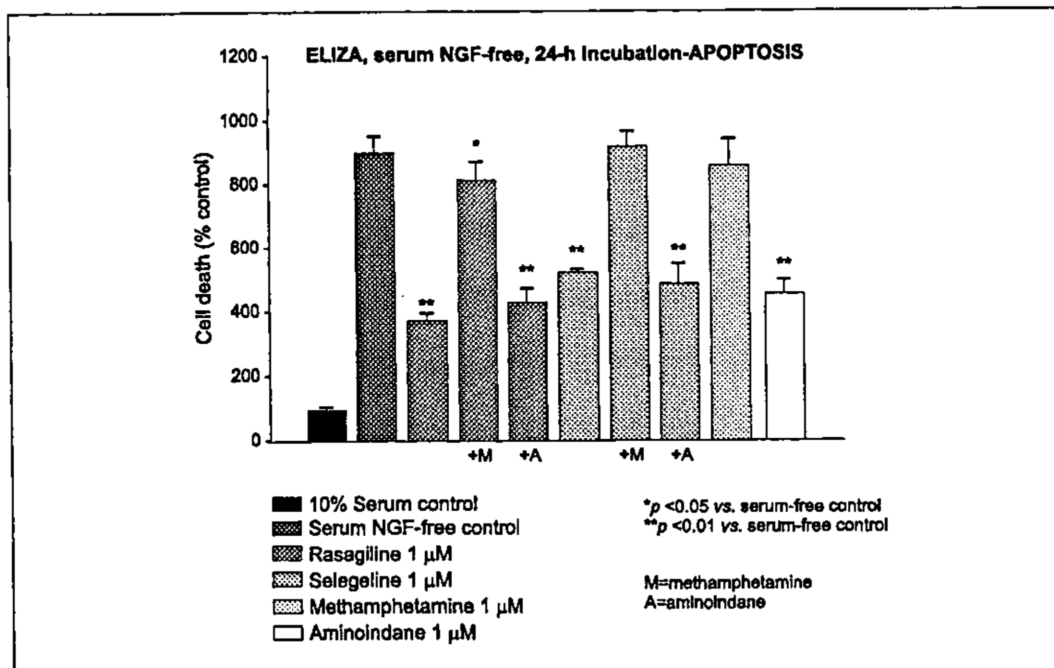

Fig. 4. The neuroprotective effect of rasagiline's metabolite aminoindan and the neurotoxicity of selegiline's metabolite methamphetamine in PC-12 cells (ref. 86).

ticed. These results may point to a possible role for rasagiline as an mGlu1 antagonist. Support for this has come from cDNA microarray gene expression and proteomics with chronic rasagiline treatment in mice. Rasagiline significantly decreases the expression as well as the proteins of the glutamate and AMPA receptor in the mid brain of mice (96). Furthermore, rasagiline's metabolite aminoindan, unlike selegiline's metabolite methamphetamine, not only has no toxic effects, but also has neuroprotective activity under the conditions studied. Its generation *in vivo* may have an additive neuroprotective effect to that of rasagiline. As one of a few compounds identified for neuroprotective studies in Parkinson's disease (64), rasagiline may have the added advantage of neuroprotective properties arising from both its nontoxic metabolite and the parent compound. The involvement of the protein kinase C (PKC)-dependent mitogen-activated protein (MAP) kinase pathway in the neuroprotective activity of rasagiline in the absence of serum and nerve growth factor has recently been demonstrated. The ERK inhibitor PD98096 and the PKC inhibitor GF109203X prevent the neuroprotective activity of rasagiline normally observed in PC-12 cells in the absence of serum and nerve growth factor (86) and will be discussed later.

Mitochondrial permeability transition pore and neuroprotective activity of rasagiline

Mitochondria are potent integrators and coordinators of cell death and survival. Apoptosis and necrosis are modes of cell death that play an integral part in a variety of biological processes, and their demise has been implicated in variety of neurodegenerative diseases, including Parkinson's, Alzheimer's and Huntington's diseases, as well as in non-neurodegenerative diseases such as cardiovascular disease and diabetes (for reviews, see refs. 97–101). The participation of mitochondrial-induced apoptosis in, for example, neuronal and cardiovascular disease-related cell death has not been fully established and therefore remains a controversial subject. Nevertheless, mitochondrial-induced apoptosis has been a target for study of toxin-induced cell death and neuroprotection in progressive loss of neurons and cytoprotection in cardiovascular diseases with variety of pharmacological agents.

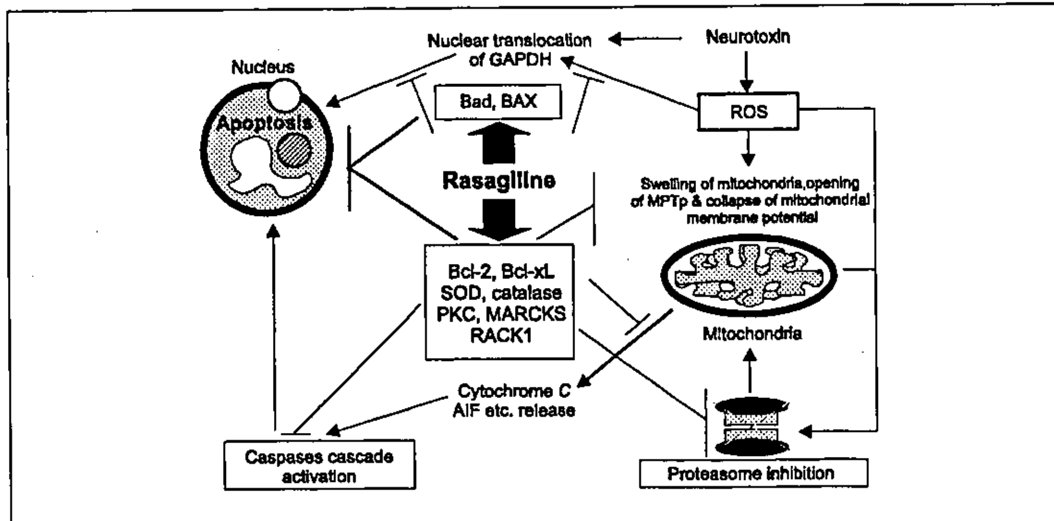

Fig. 5. The mechanism by which various neurotoxins induce neurotoxicity- and apoptosis-induced neuronal death.

The initial phase of apoptosis is triggered in response to an induction phase resulting from a variety of initial insults, including xenobiotics, parkinsonism endogenous (*N*-methyl-(*R*)-salsolinol) and exogenous (6-hydroxydopamine and *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine [MPTP]) neurotoxin radiation, oxidative stress, etc. This results in an accompanying change in mitochondrial membrane permeability and a decline in mitochondrial membrane potential ($\Delta\psi m$), swelling of the mitochondria and opening of the mitochondrial permeability transition pore (MPTp) complex, inhibition of the ubiquitin-proteasome complex, release of mitochondrial cytochrome *c* and activation of caspases, especially caspase 3, resulting in cell death by apoptosis (Fig. 5). Mitochondrial permeability transition pores play a central role in the induction and prevention of apoptosis-induced cell death and consists of mitochondrial multiprotein complex, the exact nature of which is not yet fully known. However, it is known to include porin, hexokinase, peripheral benzodiazepine receptor, creatine kinase and cyclophilin D (Fig. 6).

The direct involvement of mitochondrial permeability transition pores in apoptotic-induced death of mammalian cells, including neurons, in cell culture and *in vivo* has been well documented for a variety of agents. The important role of mitochondrial permeability transition pores is also supported by the findings that the mitochondrial permeability transition pore complexes, particularly VDAC (voltage-dependent anion channel) and ANT (adenosine nucleotide translocase), are direct functional targets for the Bcl-2 family of proteins (Fig. 6). Mitochondrial phase function is controlled by oncogenes and antioncogens of the Bcl-2-Bax family. Antiapoptotic members (*e.g.*, Bcl-2, Bcl-Xl, etc.) stabilize mitochondrial permeability transition pores, while pro-apoptotic members (*e.g.*, Bax, Bak, Bad and Bid) promote and increase mitochondrial membrane permeability (for reviews, see refs. 97–101). For example, we have shown that in response to the endogenous dopaminergic neurotoxin, *N*-methyl-(*R*)-salsolinol (SIN-1 peroxynitrite donor and 6-hydroxydopamine not shown), in the integration phase, the following responses occur in SHSY-5Y neuroblastoma cell mitochondria: a loss of $\Delta\psi m$, swelling of the mitochondria matrix (Fig. 7) and oxidative stress, and opening of mitochondrial membrane permeability (Fig. 8). Pretreatment of these cells with rasagiline prevents these effects (Fig. 6–8). In addition, there is inhibition of the ubiquitin–proteasome system by the neurotoxin, resulting in release of toxic intermembrane proteins such as cytochrome *c*, which rasagiline inhibits (68, 69). These responses are followed by a complex set of events which include activation of pro-caspases and caspase 3 activation (which rasagiline prevents [66, 67]), resulting in nuclear chromatin condensation, DNA fragmentation and changes in the plasma membrane that culminate in the death of SHSY-5Y

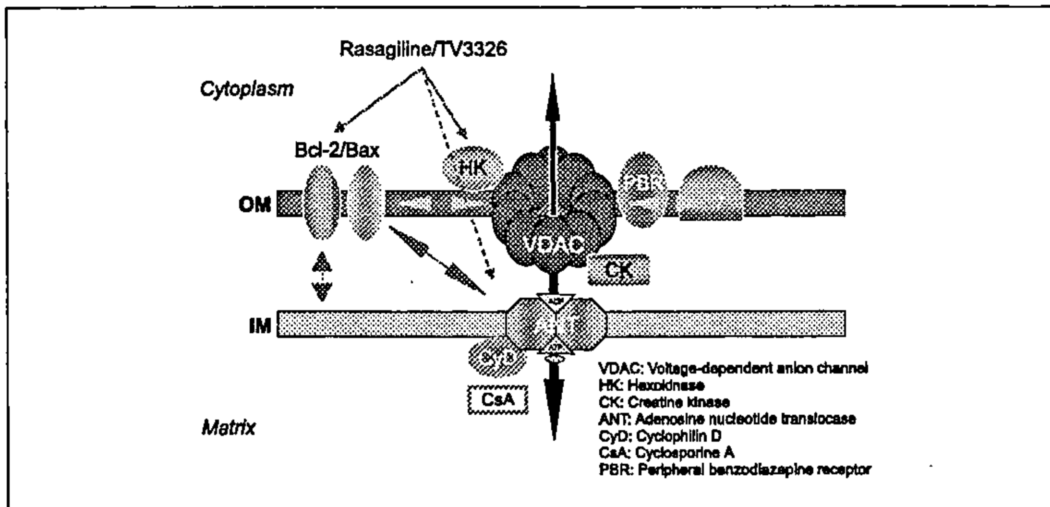

Fig. 6. Sites of rasagiline and TV3326 neuroprotective actions in preventing neurotoxin (sin-1, 6-hydroxydopamine and *N*-methyl-(*R*)-salsolinol) induced opening of mitochondrial permeability transition pore (MPTp) (ref. 53).

cells (Fig. 5). Rasagiline-induced neuroprotection exhibited in response to *N*-methyl-(*R*)-salsolinol is also seen with a number of other neurotoxic events and compounds that induce neuronal death (Table I, Fig. 5). This has provided the first evidence that propargylamines and rasagiline antiapoptotic drugs regulate the anti- and pro-apoptotic signaling pathway in mitochondria. The ability of rasagiline to inhibit *N*-methyl-(*R*)- salsolinol-induced collapse of mitchondrial membrane potential ($\Delta\psi m$), the swelling of mitochondria (Fig. 7) and mitochondrial membrane permeability opening (Fig. 8) in SHSY-5Y cells is associated with activation of antiapoptotic Bcl-2 family proteins. Evidence for this has come from the studies demonstrating that rasagiline increases mRNAs and proteins of Bcl-2 and Bcl-Xl, while decreasing those of Bax and Bad in SHSY-5Y and PC-12 cells (submitted for publication). Furthermore, Bcl-2-overexpressed SHSY-5Y cells behave identically to rasagiline-treated cells by being resistant to neurotoxicity with *N*-methyl-(*R*)-salsolinol (SIN-1 and 6-hydroxydopamine not shown) (Figs. 6, 7) (53, 102). The neurotoxicity induced by *N*-methyl-(*R*)-salsolinol in SHSY-5Y cells, resulting in the inhibition of the ubiquitin-proteasome system, is associated with cytosolic translo-

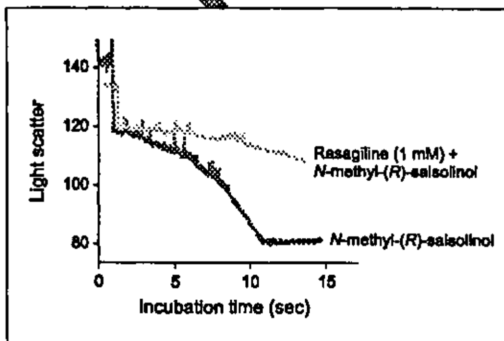

Fig. 7. Prevention of rat liver or brain mitochondrial swelling by rasagiline as induced by neurotoxin *N*-methyl-(*R*)-salsolinol (ref. 102).

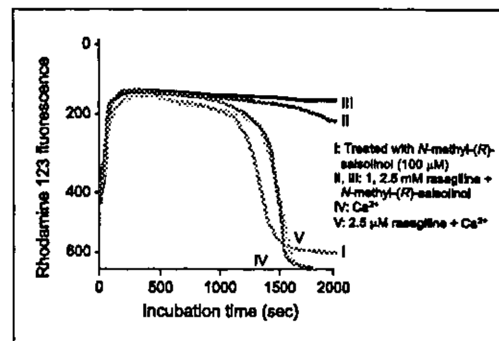

Fig. 8. Prevention of the permeability transition by rasagiline in isolated mitochondria as induced by *N*-methyl-(*R*)-salsolinol, but not of that induced by Ca^{2+} (ref. 102).

cation of pro-apoptotic GAPDH. However, it is not known whether the two are linked or independent pathways leading to apoptosis and cell death. Both rasagiline- and Bcl-2-overexpressed cells prevent these processes (68, 69). Further support for the participation of Bcl-2 family proteins in the neuroprotective activity of rasagiline has come from cDNA microarray gene expression in rat striatum with chronic rasagiline treatment. In these studies, cell survival (Bcl-2, Bcl-xl, AKT, PKC) and death (FAS, Bax and Bad, JNK) are respectively up- and downregulated (96, 103).

Structure-activity relationship studies with propargylamines have indicated that the propargyl moiety is crucial for the neuroprotective activity (75, 104–106), since although the aminoindan metabolite of rasagiline is not antiapoptotic, in certain other circumstances it has neuroprotective activity, as in the case of serum withdrawal in partially neuronally differentiated PC-12 cells (81, 86). The mechanism responsible for the neuroprotective action of rasagiline in some respects resembles that of cyclosporine A and immunophilins, which have been shown to have neuroprotective activity against a variety of neurotoxins, including *N*-methyl-(*R*)-salsolinol and MPTP (107). However, rasagiline is unable to fully suppress the Ca^{+2}-induced mitochondrial membrane permeability opening, as seen with BCL-2 overexpression (Fig. 7) and with the action of bonkrekic A and propargylamine, all of which target respective components of the mitochondrial permeability transition pore complex. Nevertheless, in many respects the neuroprotective response of SHSY-5Y cells to rasagiline is very similar to that of cells with overexpressed Bcl-2 (68, 102, 108). Such cells are also resistant to apoptosis by neurotoxins such as *N*-methyl-(*R*)-salsolinol, and there is no collapse of $\Delta\psi m$, no inhibition of the ubiquitin-proteasome system, no release of cytochrome *c* and no GAPDH translocation from the cytoplasm to the nucleus in response to the neurotoxin (Fig. 5) (68, 102, 108). Our current studies are directed at identifying the target protein(s) upstream and determining at which VDAC protein site rasagiline acts upon the mitochondria (68, 69). Preliminary proteomic studies suggest that upstream rasagiline acts to downregulate FAS and FAS receptor while upregulating PKC (109).

Amyloid precursor protein processing activity of rasagiline

Activation of PKC-dependent MAP kinase pathway

PKCs, which are abundant in neuronal tissues (110), are involved in neuronal survival and functions of neuronal trophic factors (111, 112). They are critical in the formation and consolidation of different types of memory (113), suggesting a crucial role for PKC in the aberrant signal transduction occurring in Parkinson's and Alzheimer's disease brain (114). Indeed, several reports document a deficit in PKC isoform levels in Alzheimer's disease (115–117), which might lead to a reduced responsiveness of brain tissues to growth factors and neurotransmitters, including acetylcholine (114, 118). Furthermore, a defect in PKC activation in Alzheimer's disease 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 (119). *In vitro* studies have established the involvement of PKC and PKC-coupled receptors in the nonamyloidogenic - secretase pathway of the amyloid precursor protein (APP) cleavage (120–123). There is also evidence for the regulation of secretory APP processing by PKC-dependent mechanisms under *in vivo* conditions (124–126).

Recently, a series of novel analogues was synthesized with a carbamate cholinesterase inhibitory moiety in the aminoindan structure of rasagiline, with the purpose of preserving its neuroprotective activity and also to inhibit acetylcholinesterase in order to increase cholinergic transmission (127, 128). These analogues were designed for use in the treatment of Alzheimer's disease. The *R*-enantiomer of these compounds, TV3326 ([*N*-propargyl-(3*R*)aminoindan-5-yl]-ethyl methyl carbamate), inhibits cholinesterase and MAO and improves memory impairment in scopolamine-treated rats. Its *S*-isomer, TV-3279, which is also a cholinesterase inhibitor, has no MAO inhibitory activity, but it does have similar action in preventing scopolamine impairment in the spatial learning test (127, 128). These compounds retained the neuroprotective activities of rasagiline in partially differentiated PC-12 cells deprived of serum and nerve growth factor, as well as *in vivo* (44, 81, 127). They also prevented AβAPP-induced PC-12 cell death (104). In addition, rasagiline, together with its anti-Alzheimer's drug derivatives TV3326 and TV3279, induces the release of the nonamyloidogenic α-secretase form of soluble APP (sAPP) from SH-SY5Y neuroblastoma and PC-12 cells by mitogen-activated protein kinase (MAPK)- and PKC-dependent mechanisms (104, 129). These processes have been shown to be directly depen-

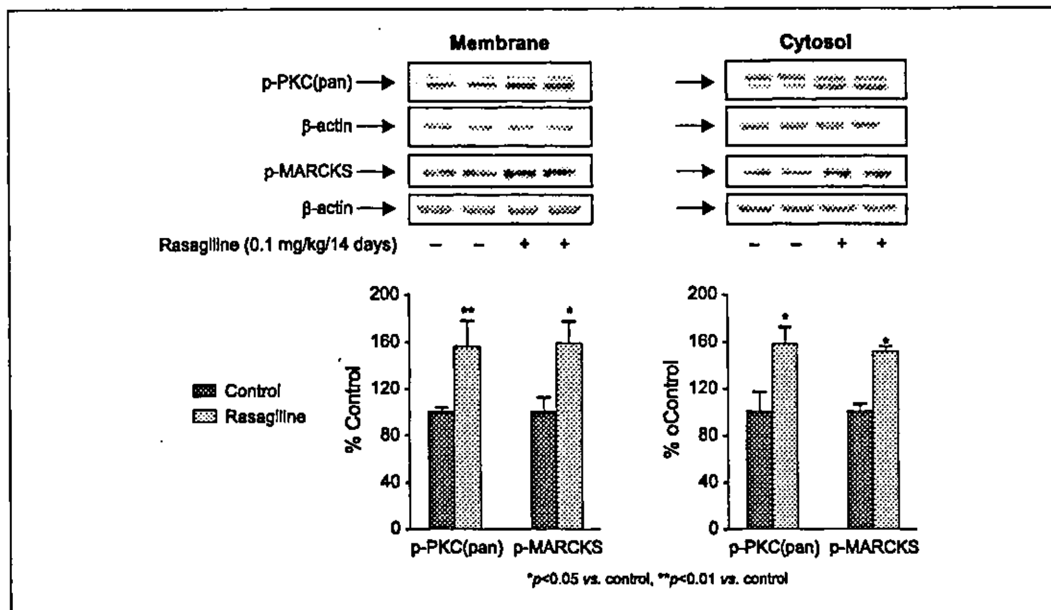

Fig. 9. Effects of rasagiline on p-PKC(pan) and p-MARCKS levels in mouse hippocampus after chronic treatment with rasagiline (ref. 130).

dent on the propargylamine moiety of rasagiline and its derivative, since propargylamine itself is as effective in these mechanisms (102). Indeed, the neuroprotective activity of these compounds is also dependent on the propargylamine moiety (81, 104–106). Considering these findings, the effect of rasagiline and its derivatives on the regulation of PKC-dependent mechanisms and APP processing under *in vivo* conditions was investigated.

Evidence has been provided that rasagiline and its cholinesterase inhibitor derivatives TV3326 and TV3279 induce significant increases in p-PKC levels and in the expression of α and ε PKC isoforms in the mouse and rat hippocampus (86). Additionally, rasagiline treatment significantly elevated the levels of p-MARCKS, a major substrate for PKC, as well as levels of RACK1 (Fig. 9). Similar results have been obtained with propargylamine alone, indicating that the PKC activation by rasagiline and its derivatives is the pharmacological property of the propargylamine moiety (104, 130).

Studies investigating the role of the PKC family in the regulation of cell death have suggested that activation of PKC can prevent apoptosis via two main survival pathways: 1) the antiapoptotic protein BCL-2; and 2) the MAPK/extracellular regulated kinase (ERK) cascades. PKC can phosphorylate Bcl-2 at a site that increases its antiapoptotic function (131), and overexpression of PKC results in increased expression of Bcl-2 (132, 133). Furthermore, MAPK/ERK cascades, which have been shown to inhibit apoptosis in a number of systems, can be activated by PKC. For example, PKC phosphorylates and activates raf-1, an upstream kinase in the MAPK/ERK pathway (134), and pharmacological inhibition of MAPK/ERK signaling blocks phorbol ester-induced protection of neuronal cells against glutamate toxicity (135). Indeed, both of these pathways have been identified in the neuroprotective mechanism of action of rasagiline *in vitro*, where treatment in PC-12 cells with the ERK inhibitor PD98059 and the PKC inhibitors GF109203X and calphestin c (submitted for publication) prevented the neuroprotective activity of rasagiline (and its derivatives, unpublished data) in serum- and nerve growth factor-free system-induced apoptosis (130). Rasagiline induced cytoprotective gene expression (*e.g.*, Bcl-2 and Bcl-xl), which may rescue neurons from apoptosis, as mediated by the mitochondrial death cascade, and therefore protect declining neurons in neurodegenerative disorders and aging (136). Ad-

ditionally, rasagiline dose-dependently (0.1–10 μM) increases the immunoreactivity of the phosphorylated MAPK in PC12 cells. The MEK inhibitor, PD98059, antagonized rasagiline-induced MAPK activation, indicating that MEK phosphorylated MAPK in the presence of rasagiline (104, 129). Thus, the activation of PKC by rasagiline may play a crucial role in its neuroprotective activity, which also involves the regulation of Bcl-2/Bcl-Xl/Bad and Bax.

One of the major downstream substrates of PKC is the MARCKS, which has been implicated in cell motility, cell adhesion, membrane traffic and mitogenesis. PKC phosphorylates serines 152, 156 and 163 of MARCKS (137, 138), which regulates MARCKS's calcium/calmodulin binding activity and filamentous actin cross-linking activity (137, 139). MARCKS is developmentally regulated, being highly expressed in select regions of the developing and adult rat brain (140), and it plays a significant role in spatial learning processes (141). The location of MARCKS in senile plaques along with PKC and Aβ fibrils points to a possible involvement in Aβ-induced neuronal dysfunction (142). Since the phosphorylation of MARCKS has been used as a marker for activation of PKC, the effect of rasagiline on p-MARCKS levels was investigated. The results of these studies showed that p-MARCKS levels were elevated in the hippocampus of rasagiline-treated mice as compared to controls, further supporting the induction of PKC activation by rasagiline. Similar results were also obtained with TV3326 and TV3279 (86).

Owing to the reputed role of RACK1 (receptor for activated C kinase 1) in PKC-mediated events in different cellular systems, the effect of rasagiline treatment on RACK1 levels was also determined. Rasagiline treatment increased the levels of RACK1 in the hippocampus, indicating that this compound may also affect other factors controlling PKC activation. RACK1 is emerging as important in targeting activated PKC to different intracellular sites, where substrates can be phosphorylated and thus modulate PKC-dependent functions (143). In brain, RACK1 mRNA and protein show developmental changes; a parallel in RACK1 and PKC protein ontogenesis suggests that they are interdependent and involved in synaptogenesis and myelination (144). In addition, RACK1 immunoreactivity is reduced in brain cortex of the aged rat, concomitantly with an impaired translocation in PKC activity and immunoreactivity for βII, indicating that RACK1 deficit contributes to age-dependent impairment in PKC activation/compartmentalization (144). Interestingly, it has been shown that in Alzheimer's disease, a reduced level of RACK1 protein can be observed in the brain of affected patients (145), a finding that links to previous observations concerning defective PKC machinery in Alzheimer's brain and peripheral tissues (144, 146). Thus, it can be suggested that upregulation of PKC by rasagiline may maintain appropriate PKC activity, an important task in the management of Alzheimer's disease and in parkinsonian subjects with dementia pathophysiology.

In Alzheimer's disease, a growing body of evidence suggests that increased expression and/or altered processing of APP and the ensuing increase in generation of β-amyloid peptides play a central role in amyloidogensis processes (147). Thus, the observation that rasagiline markedly decreased the levels of cell-associated, full-length APP in the mouse hippocampus could be of value towards accelerating nonamyloidogenic APP processing, thereby reducing β-amyloid levels. Since we have recently demonstrated the involvement of PKC in the release of sAPPα by rasagiline and its cholinesterase inhibitor derivatives (TV3326 and TV3279) in PC-12 cells and SH-SY5Y neuroblastoma cells (104, 129), it is possible that the mechanism by which these drugs affect APP processing *in vivo* may be related to PKC-associated signaling. Indeed, among the various signaling cascades known to participate in Alzheimer's disease pathophysiology and APP processing, PKC has received much attention. *In vivo*, it was shown that specific cholinergic lesions within the basal forebrain of rats lead to reduced secretory APP processing in the cholinergically deafferented neocortex, which strongly correlates with reduced activation of PKC-coupled M1 mAChR (125). Also, Lin *et al.* (126) observed reduced secretory APP processing in neocortex after specific cholinergic lesions of the basal forebrain and increased APP secretion (*i.e.*, less cell-associated APP but higher levels of cerebrospinal fluid secretory APP) after muscarinic agonist treatment. In an animal model of permanent hyperactivation of PKC in neocortex and hippocampus (*i.e.*, in the offspring of rats receiving *in utero* methylazoxymethanol acetate treatment), secretory APP processing was increased (124).

It is currently unknown which isoenzyme of PKC plays a major role in modulating APP processing. Nevertheless, several lines of evidence suggest the involvement of PKCε and PKC in APP processing. Thus, it was demonstrated that the EC_{50} for

PMA regulation of sAPP release was lower in Swiss 3T3 fibroblast cells overexpressing PKCα (122), and a specific inhibitor of PKCα, GO-6976, reduced constitutive and phorbol ester regulation of sAPP in human fibroblasts (148). Furthermore, in a rat fibroblast cell line, sAPP was increased after stable overexpression of PKCα and PKCε isoenzymes (149). It was also shown that blockade of PKCε activation attenuated phorbol ester-induced increase of α-secretase-derived sAPP (150). This finding further supports previous studies on brains of patients with Alzheimer's disease, where PKCε activity in the membrane fraction was reduced (117). In accordance with these findings, we have shown that chronic administration of rasagiline for 14 days causes significant increases in the protein expression of PKC isoenzymes α and ε in the mouse and rat hippocampus. These results are consistent with our recent finding demonstrating induction of PKCα and PKCε mRNAs in PC-12 cells by rasagiline (manuscript in preparation).

In summary, rasagiline, similar to its novel carbamate-containing cholinesterase inhibitor analogues, TV3326 and TV3279, reduced the levels of APP and upregulated the levels of p-PKC, PKCα and PKCε in the mouse hippocampus and in PC-12 cells (130). Furthermore, the presence of a propargylamine group in these compounds is essential for their APP processing to neuroprotective-neurotrophic sAPPα through α-secretase regulation and PKC activation-dependent neuroprotective activities (53). Moreover, studies on structure-activity relationships among rasagiline-related compounds have shown the crucial role of the propargyl moiety in these molecules with respect to these processes. This is supported by the recent observation that propargylamine itself was neuroprotective and able to regulate APP processing and MAPK phosphorylation with similar potency to that of rasagiline and its derivatives (104). Rasagiline and its derivatives TV3326 and TV3279 are now being developed as antidementia drugs in Alzheimer's disease, Lewy body disease and parkinsonian dementia.

Neurotrophic activity of rasagiline: The involvement of GDNF, nerve growth factor and NF-κB

Rasagiline increases the mRNA and protein levels of GDNF (Fig. 10) (151), in addition to those of antiapoptotic Bcl-2 and Bcl-xL, as previously reported (102) in SH-SY5Y cells in culture. Selegiline and desmethylselegiline, propargylamines structurally related to rasagiline, were previously shown to increase mRNA levels of neuroprotective proteins,

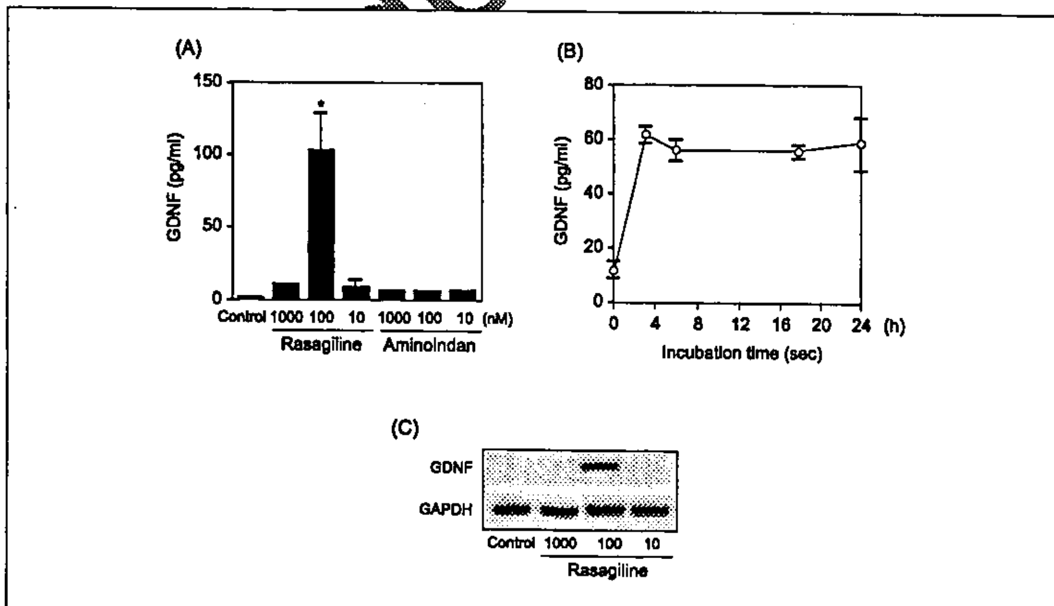

Fig. 10. Induction of GDNF synthesis and release by rasagiline in SHSY-5Y human dopamine neuroblastoma cells (ref. 151).

bcl-2, SOD, glutathione peroxides and GDNF in PC-12 cells (80). It is apparent that the activation of nuclear factor (NF)-κB by rasagiline may mediate the increase in the transcription of pro-survival genes.

In Parkinson's and Alzheimer's diseases, particular neurons deteriorate in a slow and continuous process, in which not only apoptotic, but also pro-survival factors may be activated. NF-κB may be a common regulator collecting information regarding upstream signal transduction events to determine the survival or death of the cells (152). Increased levels of NF-κB were detected in the brains of patients with Alzheimer's disease (153) and Parkinson's disease (154). Apoptosis, as induced by excitotoxicity (glutamate or NMDA) and oxidative stress, activates NF-κB (155), whereas cytotoxicity can be mediated by the activation of pro-apoptotic members of the Bcl-2 family (156). On the contrary, NF-κB has been reported to be cytoprotective against apoptosis as induced by oxidative stress and excitotoxic insults (157). These opposing results suggest that the cell types, the conditions of cytotoxic stimuli and the concomitant activation of other transcription factors may determine whether activated NF-κB promotes cell survival or cell death. The molecular mechanisms of NF-κB activation have been elucidated by Karin and Ben-Neriah (158). NF-κB is activated by IκB phosphorylation by kinase complex (I-B kinase, IKK) composed of IKK-α, -β and -γ. When the IKK complex is phosphorylated, IκB and IκBβ are cleaved at two serine residues in the *N*-terminal region and dissociated, resulting in the activation of NF-κB. Sulfasalazine, an inhibitor of IKK-α and -β (159), inhibits the rasagiline-induced NF-κB activation and the NCI 1420 1–8 induced GDNF protein simultaneously (151). These results indicate that rasagiline may activate NF-κB through the IKK pathway, resulting in GDNF synthesis. Whether rasagiline directly or indirectly affects the IKK components through activation of upstream kinases in the cells remains to be elucidated.

Rasagiline and its various derivatives, but not its aminoindan metabolite, activate PKC and Erk1/2 MAP kinase (104, 129) in a time- and concentration-dependent manner, a property directly dependent on the propargylamine moiety of these drugs, since propargylamine itself had an identical effect with the same potency (104). Activation of PKC by rasagiline is linked to its neuroprotective activity, since inhibitors of ERK and PKC prevent PKC pathway-dependent Bcl-2-induced neuroprotection, thus eliminating the neuroprotective activity of rasagiline (86, 130). This would be compatible with the recent finding indicating a link between PKC and Bcl-2. The results suggest that rasagiline targets the Bcl-2 antiapoptotic family and the protein regulating signal transduction in the MAP kinase system, as well as transcription factors. The mechanism of this these interactions is not yet fully known, but PKC activation may have a role that needs to be investigated. Although the ability of rasagiline to activate NF-κB and increase GDNF is concentration dependent, this action, as well as its ability to be neuroprotective and activate Bcl-2 and PKC, exhibits biphasic curves. Rasagiline has also been shown to exhibit an inverted U-shaped relationship in its neuroprotective function (44, 69) and in its induction of Bcl-2 (102). Tumor necrosis factor (TNF)-α, reactive oxygen species H_2O_2 and β-amyloid all activate NF-κB according to an inverted U-shaped dose-response-dependent curve. While at the low concentration, TNF-α is neuroprotective in correlation with NF-κB activation, in contrast, at the high concentration it is neurotoxic (160). The mechanism behind the inverted U-shaped type of concentration-activity relationship concerning NF-κB remains to be fully elucidated. Such phenomena are not unusual since dopamine, apomorphine, melatonin and green tea polyphenol exhibit exactly the same type of action in SHSY-5Y cells. For these compounds, it has been shown that at low concentrations, their neuroprotective activity is related to activation of the antiapoptotic bcl-2 family genes and proteins. In contrast, at high concentrations—when they are pro-apoptotic and induce cell death—they activate pro-apoptotic Bad, Bax and other cell-inducing genes (103). GDNF is now proposed as an agent for rescuing declining dopamine neurons in Parkinson's disease, as supported by its effectiveness in animal and cellular models (161). Lentiviral-delivered GDNF was reported to rescue dying dopamine neurons in a monkey model of Parkinson's disease prepared with *N*-methyl-4-phenyl 1,2,3,6-tetrahydropyridine (MPTP) (162). Infusion of GDNF protein by an implanted intracerebroventricular catheter failed to improve symptoms in parkinsonian patients (163). However, it was recently reported that the direct infusion of GDNF into the putamen of parkinsonian patients improved clinical symptoms and fluorodopa uptake to the dopamine terminal (164). These results indicate that GDNF supplemental therapy requires further technical improve-

ment for its effective administration. If rasagiline, which is currently undergoing FDA revision for approval as an antiparkinsonian drug (26), can also increase endogenous GDNF in the human brain, as shown here with SH-SY5Y cells, it may be a more practical therapy to prevent dopamine neuron cell death. In conclusion, rasagiline and related propargylamines are possible neuroprotective agents, and a part of their pharmacological action may be due to the induction of pro-survival genes through NF-κB activation, antiapoptotic Bcl-2 family protein production and inhibition of GAPDH translocation from cytoplasm into the nucleus (69). The activation of transcription factors related to antiapoptotic proteins, GDNF and bcl-2 (Maruyama *et al.*, in preparation) by propargylamines might enable us to suppress neuronal death in neurodegenerative disorders in general. Clinical trials with rasagiline are awaiting final evaluation of the efficacy data to determine the compound's ability to protect specified neurons from degeneration.

Conclusion

Pharmacologically and biochemically, rasagiline is demonstrating neuroprotective activity *in vitro* and *in vivo*. The molecular aspect of this neuroprotective property has now been demonstrated by conventional methods and by genomic and proteomic analysis to involve several factors, including downregulation of pro-apoptotic Bcl-2 family proteins that regulate the mitochondrial-dependent cell survival mechanism on the one hand, and activation of the transcription factor NF-κB and the PKC-dependent MAP kinase pathway that regulate production of neurotrophic factors, including GDNF, BDNF and nerve growth factor (86, 130, 151). Thus, rasagiline may have pharmacological activities that initiate neuroprotection, as well as neuronal plasticity related to the activation of Bcl-2 and PKC. It is apparent that Bcl-2 and PKC pathway-dependent neuroprotective and neurotrophic activities of rasagiline are closely linked, since both ERK and PKC inhibitors prevent these activities (86, 102). Whether this neuroprotective activity can be translated into the treatment of parkinsonian and Alzheimer's disease subjects remains to be determined by PET and SPECT in clinical studies. However, it should be noted that results from recent long-term double-blind controlled studies with rasagiline suggest that it may exert disease-modifying action (27).

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