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CNS Targets for multi-functional drugs in the treatment of Alzheimer's and Parkinson's diseases

Review

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Summary. Patients with mild forms of dementia and age-related memory impairment have just begun to benefit from pharmacotherapy developed over the last several years. However, current approaches do not significantly modify the course of neurodegeneration or of the aging process, and they offer limited and transient benefit to many patients. The goal of this review is to summarize new potential approaches in which molecules have been developed expressly to target multiple brain systems for the treatment of memory and cognition impairment. Some of these approaches include the development of single molecular entities that combine activity as cholinesterase inhibitors, muscarinic cholinergic M2 receptor antagonists, nicotinic acetylcholine receptor agonists, α_2-adrenergic agonists, or monoamine oxidase inhibitors. Many of the bi-functional compounds discussed have improved efficacy as cognitive enhancing agents and/or they offer potential for neuroprotection and disease modification. It is likely that syndromes such as Alzheimer's disease will require multiple drug therapy to address the varied pathological aspects of the disease. Even if the strategy of combining drugs with different therapeutic targets is workable, the development of multi-functional compounds will obviate the challenge of administering multiple single drug entities with potentially different degrees of bioavailability, pharmacokinetics, and metabolism. Also, the simplification of the therapeutic regimen for individuals with AD who have difficulty with compliance is important.

Keywords: Age-associated memory impairment, amyloid beta peptide, acetylcholinesterase, Alzheimer's disease, amyloid precursor protein, delayed matching-to-sample, monoamine oxidase, nerve growth factor, Parkinson's disease.

Diversity of neural systems important for cognition

A wide variety of clinical syndromes can manifest cognitive or memory dysfunction. These include head trauma, cerebrovascular accidents, convulsive disorders, nutritional deficits, drug-associated toxicity, etc. Although collectively these entities contribute significantly to the overall amalgamation of known memory disorders, by far the primary disease entity targeted by pharmaceutical research is Alzheimer's disease (AD). AD, which represents the most common form of dementia among individuals over 65 years of age, is now the third most expensive health care problem in the U.S. exceeded only by cancer and cardiovascular disease. It currently affects approximately 4 million Americans and imposes an annual economic burden estimated at between $80 and $100 billion. This devastating degenerative condition also inflicts an enormous emotional toll on patients, family members, and caregivers. As the geriatric population inexorably increases, the numbers of AD patients may increase to epidemic numbers (i.e., in excess of 9 million) by the middle of the twenty first century.

An additional concern of older adults is the perception that memory loss occurs as a natural result of aging. This apprehension has contributed at least in part to the enormous increase in sales of over the counter remedies and homeopathic products with claims of memory-enhancing properties. This demand from a large and ever increasing elderly population also has provided the basis for a rising interest in the development of pharmacological agents, not only for the treatment of AD, but also, for the much more common, mild cognitive decline associated with normal, non-disease aging (Green, 1995). A measurable (albeit mild) decline in cognitive function can occur as a part of healthy aging in humans which begins at some point after the fifth decade of life. The changes observed in typical (non-pathologic) aging are manifested primarily as mild deficits in declarative memory which are thought to result as a consequence of a reduction in the speed of central processing necessary for encoding and retrieval of information (Morris et al., 1991). Mild memory deficits that exceed those associated with normal aging, but that do not meet the (DSM IV) criteria for a diagnosis of dementia, have been referred to as ''benign senescent forgetfulness'' and ''age associated memory impairment'' (AAMI). Recent evidence suggests that patients with AAMI have an increased risk of developing dementia (Green, 1995), a finding that has generated considerable concern and provided the impetus for rigorous investigation.

The study of AD, particularly of the neurochemistry of the postmortem AD brain, has provided perhaps a greater level of indirect evidence for important components of cognitive and mnemonic pathways than has the study of the ''normal'' aging brain. Indeed the well known selective vulnerability of basal forebrain acetylcholine-containing neurons in AD has underscored the importance of this neurotransmitter system in memory and perhaps in other behavioral and cognitive functions affected by the disease. Among the host of degenerative processes occurring in AD, reproducible cholinergic deficits are consistently reported; they appear early in the disease process, and correlate well with the degree of dementia (Francis et al., 1999). Moreover, abnormalities in cholinergic function are frequently reported in other degenerative conditions such as Parkinson's disease (PD), diffuse Lewy body dementia and Huntington's

disease. As in AD, such cholinergic deficits often correlate with memory decline and dementia. Extensive AD-related neuropathology also is commonly found in areas normally rich in norepinephrine (locus ceruleus) and serotonin (dorsal raphe nucleus), particularly in the later stages of the disease (Engelborghs and Deyn, 1997). Findings regarding the loss of gutamatergic and certain peptidergic pathways in the AD brain also are extant in the literature (Engelborghs and Deyn, 1997; Myhrer, 1998). These findings have prompted studies designed to reveal the potential for targeting these depleted neurotransmitter systems with respective receptor agonists or other synaptic signal-strengthening drugs. Indeed, both AMPA and NMDA glutamate receptor agonists have been studied for their potential cognition enhancing actions (Weiser, 2004). In each case the potential of these compounds has been limited by the concern that they may prove neurotoxic over long term administration. Whether glutamatergic agonists prove to be clinically useful as cognition enhancing agents, one NMDA receptor antagonist, memantine, is currently approved for the treatment of AD. The drug's actions are considered to be neuroprotective, or disease modifying, rather than cognition enhancing (also see below).

The diversity of neurotransmitter substances involved in cognition is perhaps not too surprising, since it has been well known for many years that memory is represented by several distinct processes, and different types of memory are relegated to different (but sometimes overlapping) brain regions. For example components of the hippocampal formation have been implicated in mediating or processing spatial, declarative, and episodic types of memory in humans, primates, and rodents (e.g., Gilbert et al., 1998; Riedel et al., 1999; Zola et al., 2000; Vann et al., 2000; Sybirska et al., 2000). A reasonable argument has been made for the possibility that the hippocampus does not play as important a role in semantic memory (Eichenbaum, 1997; Vargha-Khadem et al., 1997), with habit learning more dependent upon the striatum (Knowlton et al., 1996; Teng et al., 2000). Also, emotional or conditioning learning processes appear to reside within the amygdala (Bechara et al., 1995). Even within working memory or episodic memory, there appear to exist separable and interacting components that may include acquisition (attention), consolidation, and retention (short and long-term); alternatively encoding, retrieval, storage, and consolidation (see Riedel et al., 1999). Certain amnestic agents such as scopolamine appear predominantly to affect the acquisition of new learning (e.g., Elrod and Buccafusco, 1988). Selectivity of action with regard to the components of memory also has been attributed to certain memory enabling drugs, even within a pharmacological class (Buccafusco et al., 1996). Thus, it seems reasonable to conclude that there are several, if not numerous, potential targets for the pharmacological treatment of memory disorders, and that drugs that promote activity within different, but interacting components of cognitive function may be expected to act additively, if not synergistically, when administered together.

Potential multiple synergistic targets for memory enhancement

In recent years, much attention has been focused on the design of palliative agents (cholinergics, nootropics, etc.) that have the ability to offer subtle

cognitive improvement. There has been much discussion as to the reason for the limitations in therapeutic efficacy noted for these classes of compounds. For cholinergic compounds demonstrated to improve the performance of cognitive tasks in animals, the potential effectiveness offered by them (cholinesterase inhibitors and direct cholinergic receptor agonists) in humans can be limited by the appearance of central and peripheral side effects. The premise that high selectivity and high potency are the most desirable properties for a new therapeutic agent may not be the case for many drugs designed to treat brain disorders. For example, in PD activation of both D1 and D2 striatal dopaminergic neurons may be necessary for maximal drug efficacy in reducing motor symptoms. Also in the treatment of major psychoses the most useful classes of agents are proving to be those 'atypical' drugs that often exhibit low potency and little selectivity. Similar pharmacological opportunities are available for AD drugs as well. As discussed in the preceding paragraphs, multiple neurotransmitter systems are affected to varying degrees in AD. Both noradrenergic neurons and cholinergic neurons have been shown to play a role in different components of learning and memory in rats. It may require combined therapy with adrenergic agonists such as clonidine and cholinergic agonists such as acetylcholinesterase (AChE) inhibitors to fully reverse the cognitive defects resulting from combined lesions of adrenergic and cholinergic neuronal pathways (Haroutunian et al., 1990). One other example of the concept of synergistic actions of different drug classes on memory-related task performance is a report in which the muscarinic M1-preferring receptor agonist, milameline, was shown to augment the ability of the AChE inhibitor tacrine to reverse a scopolamine-induced decrement in efficiency of maintaining a continuous-performance task by rhesus monkeys (Callahan, 1999). More recently it has been reported that the cognitive enhancement produced by cholinergic muscarinic agonists may involve septohippocampal GABAergic and hippocampal glutamatergic neurons (Wu et al., 2000). The potential for combining drugs acting on the acetylcholine and glutamate systems is enhanced with the advent of the low affinity NMDA receptor antagonist memantine. This compound may prevent the excitatory amino acid neurotoxicity suggested to accompany AD without interfering with the actions of glutamate required for learning and memory (Kornhuber et al., 1989; Doraiswamy, 2002). Recent clinical trials have indicated that memtantine may improve cognition and result in the early improvement in behavior in AD (Areosa and Sherriff, 2003). Presently memantine is the only agent approved in the U.S. for the treatment of moderate to severe AD, and the drug is being prescribed in conjunction with cholinesterase inhibitor therapy (Tariot et al., 2004). Clearly this approach represents an important proof of concept related to this review, even though the two effects, cognition enhancement and neuroprotection are not combined in a single drug entity.

Many drugs and other natural substances derived from a wide variety of chemical and pharmacological classes have been shown to improve memory-related task performance in animals and humans. The clinical use AChE inhibitors is likely to continue for some time into the future, and this pharmacological class continues to represent one of the most effective drugs tested in animal models (Buccafusco and Terry, 2000); and the use of AChE inhibitors

may benefit quite dramatically from the addition of other pharmacological classes of cognitive-enhancing drugs. Our first indication that combinations of drugs might prove useful as a therapeutic approach to improving memory-related task performance was derived from one of our earlier studies in which we attempted to block the improvement in DMTS task efficiency to nicotine with the antagonist mecamylamine (Elrod et al., 1988). Generally responsiveness to nicotine is highly individualized with a narrow therapeutic window. For nicotine-induced cognitive enhancement, higher doses may be associated with side effects that could interfere with task motivation. In our initial study with mecamylamine (which was used to confirm that central nicotinic receptors mediated the positive mnemonic response to nicotine) we used the quaternary nicotinic antagonist hexamethonium to control for the potential peripheral actions (mainly ganglionic blockade) of mecamylamine on subjects performing the delayed matching-to-sample task. When the monkeys were pretreated with hexamethonium, the nicotine-induced improvement in average task efficiency was further enhanced across all delays (although the effect was not statistically significant). Thus, it may be possible to expand the therapeutic window of certain agents like nicotine by preventing peripheral side effects with the use of low levels of peripheral nicotinic receptor blockade. Along these lines, it is somewhat perplexing as to why low doses of selective peripherally-acting muscarinic antagonists such as methylatropine or glycopyrrolate have not been used in combination with cholinesterase inhibitors to help limit the latter drugs' side effects.

Targeting brain AChE and α_2-adrenergic receptors

In addition to this heuristic approach to combination therapy, we also considered the possibility of pharmacologically exploiting at least two targets, central α_2-adrenergic receptors (with clonidine) and AChE with physostigmine (Terry et al., 1993). Clonidine may target α_2-adrenergic receptors in the prefrontal cortex to evoke a moderate level of task improvement (Jackson and Buccafusco, 1991). From a different perspective there may be another rationale for considering that combined therapy may be superior to monotherapy. We have reported that clonidine is a potent inhibitor of the biosynthesis and the release of acetylcholine within specific brain regions (particularly in hypothalamic and hindbrain regions) in the rat and that the drug can inhibit the expression of cholinergic signs of toxicity to physostigmine and other cholinesterase inhibitors (see Buccafusco, 1992). However, clonidine is only weakly effective in inhibiting cholinergic function within higher brain regions, presumably containing sites more relevant to the cognitive enhancing actions of AChE inhibitors (Buccafusco, 1984). We tested the possibility that combined treatment with clonidine and physostigmine could result in enhanced effects on delayed matching-to-sample performance (DMTS) accuracy by mature adult and aged macaques.

One of the most obvious effects of adding 0.5 μg/kg clonidine to the physostigmine regimen was that the animals were able to tolerate much higher doses of physostigmine. The individualized optimal (''Best'') dose of physostigmine was determined for each animal as that dose which provided the greatest improvement in task accuracy averaged over the entire 96-trial session.

The Best Doses determined for physostigmine alone ranged from 5–40 μg/kg (mean of 21.4 ± 4.5 μg/kg). Best Doses determined for physostigmine in the presence of 0.5 μg/kg clonidine ranged from 10–60 μg/kg (mean of (40.0 ± 6.9 μg/kg) – almost a 2 fold increase. Despite the fact that doses used for physostigmine were maximal for each animal, when the two drugs were combined, a further improvement in performance was obtained. In fact, for the combination regimen, on the day after administration, performance accuracy continued to be elevated relative to baseline. Therefore, in this example the following factors appear to contribute to the enhancement of task performance in the combination: 1) a widening of the therapeutic window, most likely reflecting a reduction in AChE inhibitor-associated side effects; 2) the targeting of separate neural substrates that each play a role in cognitive function; 3) the addition of clonidine extended the regimen's overall duration of action possibly through a unique pharmacodynamic action. In this particular study we used a fixed dose of clonidine previously determined to be optimal when used alone. It is possible that additional improvement might be obtained with further optimization of the regimen. The potential interactions of clonidine and physostigmine with respect to central cholinergic neurons are diagrammed in Fig. 1A.

Though it might be considered that the potential for side effects doubles when a drug acts on two therapeutic targets, in the case of clonidine and physostigmine, these are mutually antagonistic in terms of cardiovascular side

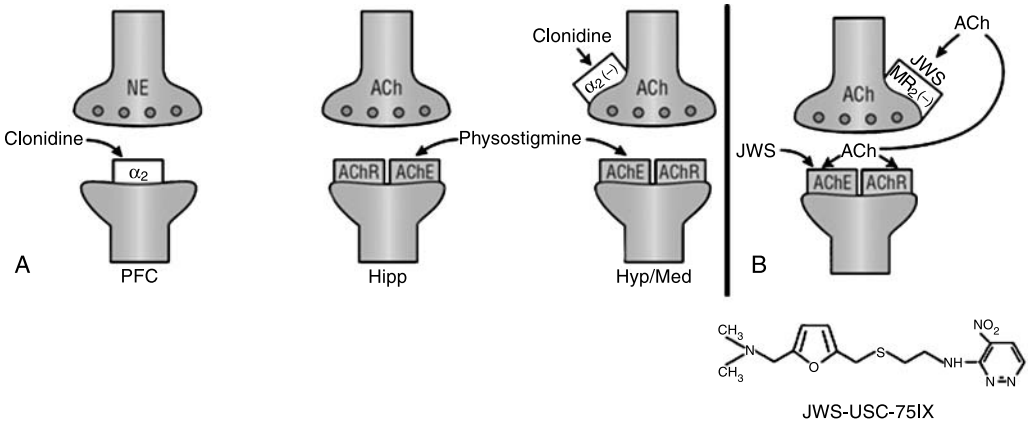

Fig. 1. Schematic representation of potential mechanisms for enhancing the efficacy of memory agents. **Panel A**: The norepinephrine (NE) agonist clonidine can improve cognition through stimulation of α_2 receptors expressed on prefrontal cortical neurons (PFC). Addition of the acetylcholinesterase (AChE) inhibitor physostigmine could result in added improvement through potentiation of acetylcholine (ACh) neurotransmission within the hippocampus. However, cholinergic neurons located in hypothalamic (Hyp) and medullary (Med) autonomic centers participate in mediating side effects of physostigmine. These neurons (but not hippocampal neurons), however, express α_2 receptors that when activated (by clonidine) inhibit ACh release, thus limiting side effects. **Panel B**: JWS-USC-75IX (JWS) is a memory-enhancing compound with dual properties. The drug is a potent AChE inhibitor, but also it is an ACh receptor (AChR) antagonist selective for muscarinic receptor subtype 2 (MR_2). Thus, JWS enhances cholinergic neurotransmission, but at the same time it inhibits normal feedback inhibition mediated through MR_2 which can limit the effects of AChE inhibitors

effects at least as they occur in experimental animals (see Buccafusco, 1992). In one study wherein the two drugs were administered to AD patients in a clinical safety study, there were no adverse cardiovascular effects noted (Davidson et al., 1989).

Targeting brain AChE and M2 muscarinic receptors

JWS-USC-75IX (3-[[[2-[[5-dimethylaminomethyl)-2-furanyl]methyl]thio]ethyl] amino]-4-nitropyridazine) is a relatively potent AChE inhibitor, but it also exhibits high affinity antagonism for the muscarinic M2 muscarinic cholinergic receptor. As AChE inhibitors have the potential of limiting their own actions through acetylcholine-induced feedback inhibition (mediated *via* activation of presynaptic M2 receptors), it was reasoned that M2 receptor antagonism could result both in the enhanced release of acetylcholine, and mitigation of the AChE inhibitor-induced feedback inhibition. JWS-USC-75IX was shown to improve the performance of rats in three different memory-related tasks, and in one of these, a delayed discrimination task, the drug was shown to exhibit repeatable improvements without the development of tolerance. The task was developed so that we could employ an operant paradigm (not unlike the primate delayed matching-to-sample task) in rats. JWS-USC-75IX also exhibited a marked safety profile relative to drugs acting only to inhibit AChE (Terry et al., 1999). The potential sites of action and their interactions are diagrammed in Fig. 1B. As with the discussion above concerning the potential cardiovascular side effects of a physostigmine plus clonidine regimen, the combination of cholinesterase inhibition and M2 receptor blockade might be expected to be mutually advantageous, though the possibility has not been tested clinically.

At this point it is appropriate to point out that all efforts to combine multiple actions in one molecule have not met with success. An example is the compound RS66331 which (neurochemically) exhibits the properties of a $5HT_4$ agonist and a $5HT_3$ antagonist. Both properties have been associated with enhanced release of brain acetylcholine (Cassel and Jeltsch, 1995). We studied this compound in aged rhesus monkeys and compared its effectiveness with that produced by individual administration of a $5HT_4$ agonist and a $5HT_3$ antagonist, both of which were demonstrated previously to enhance task performance in the same subjects. Rather than this combination of properties imbuing RS66331 with augmented memory-enhancing action, the effectiveness of the drug proved to be similar to that produced by the $5HT_3$ antagonist RS56812, but it was considerably reduced in effectiveness compared with the $5HT_4$ agonist RS17017 (Buccafusco and Terry, 2000). However, RS66331 was developed prior to our work with the individual compounds. There are many reasons for the failure of compounds to achieve expectations in memory paradigms, however, this may be one case wherein the information derived from the combined administration of various dose-regimens of RS56812 and RS17017 may have alerted us to the possibility that this is not a useful neural target combination, or to the possibility that different proportions of relative receptor activity were needed as compared to that inherent in RS66331.

Targeting brain monoamine oxidase (MAO) and cholinesterase

Relative to drugs with cognitive enhancing actions, much less work has been directed at the development of neuroprotective drugs for the treatment of AD. In some respects there is a better understanding of nigro-striatal dopaminergic neurodegeneration and neuroprotection mechanisms in PD because of the availability of relatively appropriate models. The mechanisms that may be involved in the process of neurodegeneration, particularly in AD, include oxidative stress, inflammatory processes, and accumulation of iron at the site of neurodegeneration. As such, antioxidants, MAO-B inhibitors, non-steroidal anti-inflammatory drugs and iron chelators have been suggested to exert neuroprotective actions, and they have been incompletely examined in AD. The MAO-B inhibitors selegiline and rasagiline (Birkmayer et al., 1975; Youdim, 1989; Foley et al., Parkinson Study Group, 2002 and 2004) are anti-Parkinson's (anti-PD) drugs that have warranted more scrutiny as a consequence of their neuroprotective activity in vitro (neuronal cell cultures) and in animal studies. The established co-morbidities of (1) AD with extrapyramidal features, (2) PD with dementia, and (3) both AD and PD with depression stimulated the development of a series of novel bi-functional drugs possessing the MAO inhibitory activity and neuroprotective activity exhibited by anti-Parkinson drugs selegiline or rasagiline (Weinstock et al., 2000a, b, 2001, 2003; Youdim et al., 2001a, b; Sterling et al., 2002) along with the ability to inhibit AChE (Sterling et al., 2002). For these studies the structural requirements for anti-PD activity as exhibited by rasagiline and selegiline, and the structural requirements for cholinesterase inhibition exhibited by rivastigmine was initially considered (Sterling et al., 2002; Weinstock et al., 2003).

Introduction of a carbamate moiety into the pharmacophore of rasagiline molecule (Fig. 2) resulted in almost complete loss of brain MAO-B inhibitory activity for ladostigil (TV3326) and TV3279 as determined in vitro. However, chronic oral administration (12.5–76 mg/kg) in rats, mice and rabbits showed that ladostigil (TV3326), but not TV3279, is a CNS-selective inhibitor of MAO-A and MAO-B, with little inhibition of liver and small intestine MAO (Weinstock et al., 2000a, b; Sagi et al., 2003). The possibility that ladostigil was serving as a prodrug in the brain to generate an active MAO inhibitory metabolite(s) is supported by the results of animal studies where, in contrast to the first order kinetic recovery of MAO activity from inhibition by rasagiline, TV3326 exhibited a biphasic action (Sagi et al., 2003). Also, an active metabolite of TV3294 has been identified. Both ladostigil and TV3279 inhibit AChE and butyrylcholinesterase, but with a slower time course and with slightly reduced effectiveness compared with rivastigmine. ladostigil is 100 times more potent against butyrylcholinesterase than it is against AChE. The cholinesterase inhibitory activity of these drugs is consistent with their dose-related (12–26 mg/kg) antagonism of the spatial memory deficits induced by scopolamine in rats, indicating that they were able to increase brain acetylcholine levels sufficiently to compete with scopolamine for muscarinic receptors sub-serving memory (Weinstock et al., 2000b).

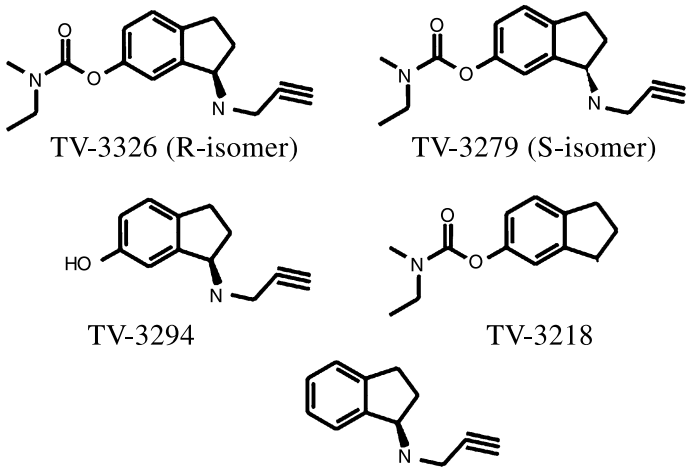

Fig. 2. Structures of cholinesterase-monoamine oxidase inhibitors, ladostigil (TV3326) and TV3279 derived from rasagiline and rivastigmine. TV3294 and TV3218 are metabolites of ladostigil which respectively monoamine oxidase and cholinesterase inhibitors (adapted from Youdim et al., 2003)

Neuroprotective – antiapoptotic action of ladostigil (TV3326)

The primary rationale for developing this class of bi-functional drugs was to combine the dopaminergic (anti-PD) and cholinergic (anti-AD) activities associated respectively with rasagiline and rivastigmine, with the neuroprotective effectiveness of rasagiline in a single molecule (Youdim and Weinstock, 2002). ladostigil exhibits both MAO inhibitory and anti-cholinesterase activities, although the drug's optical S-isomer TV3279 possesses only anti-cholinesterase activity. The profile of the neuroprotective activity of ladostigil as compared with rasagiline indicated that, to a large extent, ladostigil mimics the established neuroprotective profile of rasagiline. Both rasagiline and TVP1022 prevent neuronal damage caused by closed head injury in mice (Huang et al., 1999), focal ischemia (Speiser et al., 1998), and cytotoxicity in cultured PC12 cells induced by growth factor withdrawal (Youdim et al., 2001a) or glucose-oxygen deprivation (Abu-Raya et al., 2002). They also prevent apoptosis induced by the neurotoxins N-methyl-R-salsolinol, 6-hydoxydopamine, the peroxynitrite donor, SIN-1, and by aggregated Aβ amyloid peptide (Aβ) in SHSY-5Y neuroblastoma cells. Rasagiline and TVP1022 (0.10 nM–1 mM) prevent the loss of intact nuclei normally observed in partially-differentiated PC12 cells after serum and NGF withdrawal. This cytoprotective activity is related to their anti-apoptotic action, since the drugs significantly diminished the percentage of cell nuclei with chromatin condensation (an index of apoptosis) over the same concentration range. The anti-apoptotic action of these compounds is dependent on the synthesis of new Bcl-2 and SOD proteins, and the response is prevented by transcriptional (actinomycin) and translational

(cycloheximide) inhibitors as measured in partially neuronally-differentiated PC12 cells. A similar effect is obtained with the racemic form of ladostigil, TV3219. *In vivo*, chronic treatment rasagiline increase SOD and catalase activities in the striatum hippocampus and cortex of rats (Carrillo et al., 2000). Thus it can be inferred that ladostigil also behaves in a similar manner to rasagiline and TVP1022 (Youdim et al., 2001a; Youdim and Weinstock, 2001). The mechanism of the anti-apoptotic effect and the identity of the proteins synthesized have not been fully determined, but they have been shown to be related to the ability of both drugs to prevent the decrease in Bcl-2 and Cu–Zn-SOD1 in response to growth factor withdrawal. In SHSY-5Y cells rasagiline induces the expression of anti-apoptotic proteins as well as the mRNAs coding for Bcl-2 and Bcl-Xl, while simultaneously decreasing the pro-apoptotic Bax and Bad proteins (Youdim et al., 2003). In fact, significant evidence exists to suggest that the anti-apoptotic activity exhibited by rasagiline and its derivatives is associated with their ability (1) to prevent the collapse of the mitochondrial membrane potential by opening of mitochondrial permeability transition pores that are part of the voltage dependent anion channel (Fig. 3); (2) to inhibit cytochrome c; and (3) to activate the caspase cascade that involves caspase 3 (Youdim et al., 2003; Maruyama et al., 2001a, b; Akao et al., 2002) (Fig. 4). The MAO-inhibitory action of rasagiline is not a prerequisite for its neuroprotective action since its optical isomer TVP1022, which exhibits poor MAO inhibitory activity, is an equipotent neuroprotective agent (Maruyama et al., 2002a, b). The neuroprotective activity of rasagiline, TVP1022 and selegiline resides in the propargylamine moiety, since propargylamine itself exerts similar

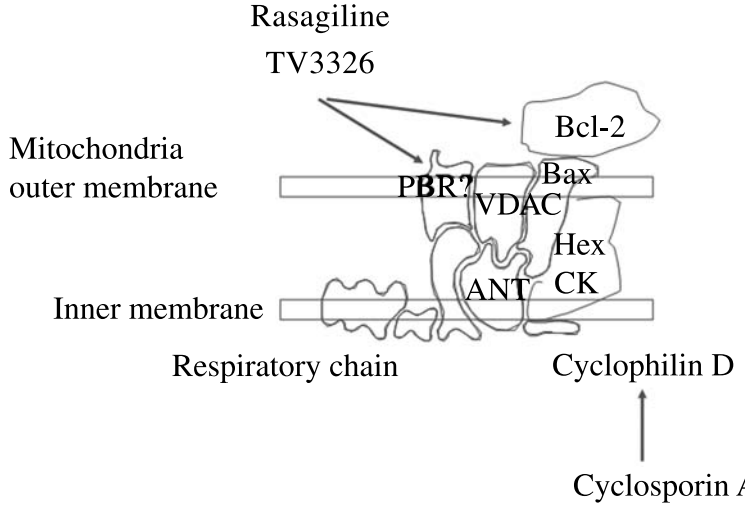

Fig. 3. Possible site of action of rasagiline and its derivatives at the mitochondrial voltage dependent anion channel (VDAC), which is part of MPT. The exact protein constituents of MPT is not known but several of the proteins, such as anti and proapoptic proteins Bcl-2 and Bax respectively; porin; *PBR*, peripheral benzodiazepine receptor; *ANT*, adenosine nucleotide translocator; *HEX*, hexokinase and *CK*, createine kinase have been identified. In a number of respects mechanism of neuroprotective action of rasagiline and its interaction with MTP resembles that of cyclosporin A, a drug with neuroprotective activity (Youdim et al., 2003)

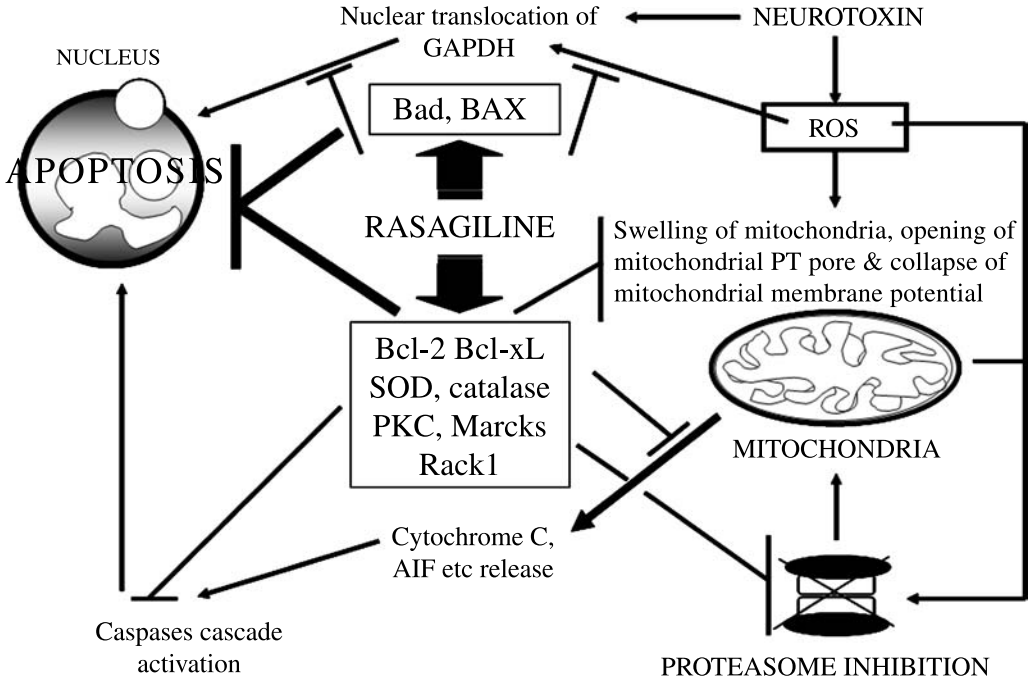

Fig. 4. Mechanism of neuroprotective-antiapoptotic action of rasagiline and its anti-Alzheimer derivative cholinesterase-monoamine oxidase inhibitor, ladostigil (TV3326). Both drugs are N-propargyl-(1R)-aminoindan derivatives with ladostigil possessing a carbamate cholinesterase inhibitor moiety. It is the propargyl moiety in these drugs that confers the neuroprotective-antiapoptoic, Bcl-2 inducing activities and PKC activating properties. Rasagiline inhibits neurotoxin (SIN-1, NM-R-Sal) initiated apoptosis in SH-SY5Y and PC-12 cells by preventing the collapse of mitochondrial membrane potential, opening of the MPT, release of ubiquitin-proteasone dependent cytochrome C and caspase 3 activation resulting in its antiapoptotic activity. It also prevents the translocation of pro-apoptotic GAPDH in these cells. Its neuroprotective activity may also depend on its activation of SOD and catalase as has been observed in vivo in various tissues including brain and heart (Youdim et al., 2003)

neuroprotective/anti-apoptotic activities (Youdim et al., 2001b; Maruyama et al., 2000, 2001a, b; Akao et al., 2002). Ladostigil and TV3279 retain the neuroprotective properties of rasagiline and TVP1022 in vivo and protects against neuronal cell culture death in response to SIN-1 and N-methyl-R-salsolinol similar to rasagiline (Youdim and Weinstock, 2001; Maruyama et al., 2003; Youdim, 2003).

Antidepressant and anti-Parkinson's activities of ladostigil and TV3279

TV3326 (but not TV3279) as a brain selective inhibitor of MAO-A and B induces significant increases in striatal, hippocampal, brainstem and hypothalamus dopamine, serotonin and noradrenaline in rats and mice (Sagi et al., 2003), a neurochemical profile is suggestive of potential antidepressant activity (Table 1). Classical antidepressant drugs such as amitriptyline and moclobemide (selective reversible MAO-A inhibitor) reduce the duration of immobility behavior in the

Table 1. Pharmacological properties of TV3326 and comparison with other cholinesterase and monoamine oxidase inhibitors

	TV3326	Riva-stigmine	Rasagiline	Clorgyline	Tranyl-cypromine
AChE inhibition	+	+	−	−	−
BuChE inhibition	+	+	−	−	−
MAO-A inhibition	+	−	−	−	−
MAO-B inhibition	+	−	+	−	−
Increase brain acetylcholine	+	−	−	−	−
Increase brain dopamine	+	−	+	−	+
Increase brain norepinephrine	+	−	−	+	+
Increase brain serotonin	+	−	−	+	+
Tyramine potentiation	−	−	−	+	+
Antidepressant action	+	−	−	+	+
Hypothermic action	−	−	NC	NC	NC
Anti-Parkinson activity	+	−	+	−	+
Neuroprotection	+	−	+	−	−

NC no change

forced swim test in rats for potential antidepressant activity (Porsolt et al., 1979; Borsini and Meli, 1988). Administration of ladostigil (26 mg/kg/day for two weeks, or 52 mg/kg for one week), inhibited brain MAO-A and B by more than 65%, and the drug significantly reduced the immobility duration to the same extent obtained after chronic treatment with amitriptyline (10 mg/kg/day) or moclobemide (20 mg/kg/day) (Weinstock et al., 2002). Ladostigil, but not TV3279, being MAO-AB inhibitor exhibits antiparkinson activity in MPTP (N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) mice model of Parkinson's disease (Sagi et al., 2003). It protects against the degeneration of nigrostriatal dopamine neurons by this neurotoxin as measured by prevention of the fall in striatal tyrosine hydroxylase and dopamine. But unlike selegiline and rasagiline it significantly increases striatal dopamine above those of control values, similar to other nonselective MAO inhibitors, since dopamine is equally well metabolized by both MAO-A and B (Youdim and Riederer, 1993).

Cognitive enhancing property of ladostigil

Recently we administered ladostigil to 7 old Rhesus monkeys well trained to perform versions of a delayed matching-to-sample (DMTS) task (Buccafusco et al., 2003). An increasing dose regimen of ladostigil was administered orally according to a schedule that allowed the animals to perform the standard DMTS task and a self-titrating version of the DMTS task each week during the study. A distractor version of the task was administered during two of the doses of ladostigil. Under the conditions of this experiment ladostigil failed to significantly affect accuracy on the standard DMTS task, however, the drug was very effective in improving the ability of subjects to titrate to longer duration delay

intervals in the titrating version of the task. This version of the task is more sensitive to age-dependent cognitive impairment than is the standard DMTS task (Buccafusco et al., 2002). The maximal drug-induced extension of the self-titrated delay interval amounted to a 36.7% increase above baseline. ladostigil also significantly improved task accuracy during distractor (interference) sessions, a measure of attention deficit. The compound was effective enough to return group performance efficiency to standard DMTS vehicle levels of accuracy. Thus, ladostigil represents a new class of drug which is potentially suitable for the treatment of AD patients who require therapies that will delay the progression of the disease, and who suffer from impaired attention, impaired memory, extrapyramidal disorder and depression. The combination of the properties attributed to an MAO inhibitor and to a cholinesterase inhibitor may derive benefit from their combined cognitive enhancing properties, as well as from the ability of adrenergic/dopaminergic receptor activations to limit the side effects of cholinesterase inhibition as discussed above for clonidine.

Amyloid precursor protein (APP) processing by ladostigil (TV3326)

One of the debated current concepts regarding the neurotoxicity associated with AD is the processing of amyloid precursor protein (APP) by the three secretases, α, β and γ, and the formation of aggregated Aβ. The potential reduction of Aβ through the administration β and γ secretase inhibitors is one approach being addressed. However, certain cholinesterase inhibitors have been shown to induce the release of neuroprotective-neurotrophic soluble amyloid precursor protein alpha (sAPPα) by selectively enhancing the action of the zinc-metaloprotease, α-secretase. Ladostigil, TV3279, rasagiline and TVP1022 induce the release of sAPPα in PC-12 and SHSY-5Y cells through activation of α-secretase. The mechanism of sAPP release has been shown to be directly linked to the propargyl moiety group on these drugs, since propargylamine itself is as effective as ladostigil, rasagiline and their S-isomer derivatives. Employing several signal transduction pathway inhibitors, it has been established that this process is mediated via the PKC-MAPK dependent pathway, as a consequence of activation of PKCα and PKCε and through ERK1/ERK2 phosphorylation (Yogev-Falach et al., 2002, 2003). In vivo, chronic oral treatment with ladostigil, TV3279 and rasagiline significantly reduced APP holoprotein in the hippocampus of rats and mice. The ability of these compounds to reduce Aβ in CHO cells and amyloid deposits in the CNS of transgenic mice that overexpress this protein is being investigated (Fig. 5).

Recent studies (Bar Am et al., 2004; Weinreb et al., 2004) have provided new insights into neuroprotective activities of rasagiline ladostigil and their derivatives the involving interaction of Bcl-2 family protein with PKC pathway. These drugs attenuate cell death as induced by serum withdrawal in rat pheochromocytoma PC12 and SH-SY5Y neuroblastoma cells inculture. Consistent with this findings are their impact on apoptosis. They the decrease serum free-induced in cleavage and activation of caspase-3 and poly (ADP- ribose) polymerase (PARP), and these effects are reversed by the protein kinase C (PKC)

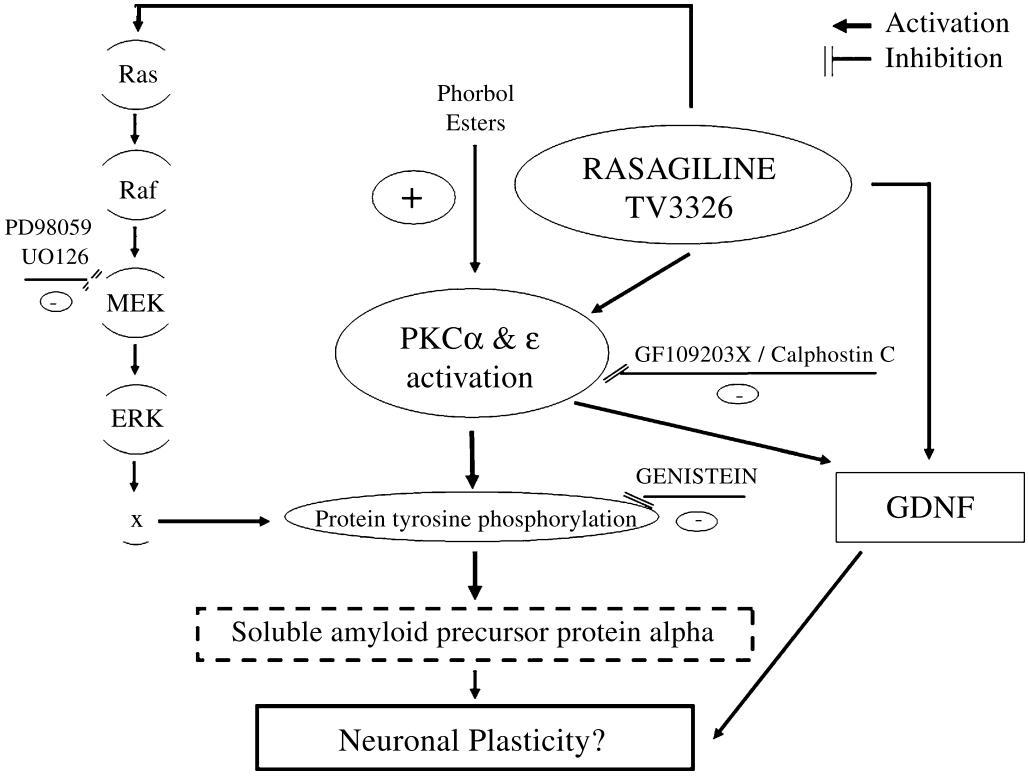

Fig. 5. Signal transduction pathways mediating the activation of PKC dependent neuroprotection and plasticity by rasagiline and ladostigil and their derivatives and N-propargylamine. These propargylamines activate PKC and MAPK pathways in a time and concentration (0.1–10 μm) dependent manner in PC-12 and SH-SY5Y cells in culture, resulting inactivation of α-secretase dependent release of sAPPa. Phorbol esters have similar action and inhibitors of PKC and MAPK pathways, as indicated, prevent rasagiline and TV3326 induced release of sAPPa. In vivo both drugs activate mice and rat hippocampal PKCα and ε promote their translocation from cytoplasm to the mitochondrial membrane. A link has now been established between PKC-MAPkinase pathway and Bcl-2 dependent neuroprotective activity of rasagiline and its derivatives, since GF109203X and PD98059 and UO126 prevent the neuroprotective activity of these propargylamine (adapted from Youdim et al., 2003; Weinreb et al., 2004; Yogev-Falach et al., 2003)

inhibitor, GF109203X. Similarly, rasagiline, N-propargylamine (and ladostigil, unpublished) decrease the serum free-induced levels of the important regulator of the cell death machinery, Bad. This effect is blocked by GF109203X, indicating the involvement of PKC in cell survival in response to these compound. Indeed rasagiline, ladostigil and their derivatives activate PKCα and PKCε, while down regulating PKCγ and δ. They also cause the translocation of PKC from cytoplasm to the membrane (Weinreb et al., 2004). Rasagiline stimulates PKC phosphorylation in a concentration-dependent manner, and induces translocation of the isoforms PKCα and PKCε to the membrane and gene expression analysis have revealed this process is associated with up regulation of PKCα and PKCε mRNA. Furthermore rasagiline prevents serum-withdrawal-induced expression of the pro-apoptotic mRNAs Bad and Bax, and the decrease in the

anti-apoptotic Bcl-xL and Bcl-w. In addition, rasagiline markedly up-regulated PKCα and PKCε gene expression and reveres the increased effect of PKCγ mRNA levels in serum deprived cells. The PKC-Bcl-2 interaction-induced neuroprotection has been identified to be the property of propargyl moiety of rasagiline, since N-propargylamine itself (Bar-Am et al., 2004; Weinreb et al., 2004) and ladostigil (unpublished) reveale a similar protective effects and mechanism of action on PKC and Bcl- family proteins as described above, suggesting the crucial role of the propargyl moiety in their neuroprotective activities (Figs. 4 and 5).

The future

The potential for multi-functional drugs for the treatment of complex neurodegenerative diseases and perhaps even for the treatment of age-associated memory impairment has already been partially realized. The AChE inhibitor galantamine is in widespread use for the treatment of the symptoms of AD. Galantamine may offer additional potential for disease modification (neuroprotection) relative to its predecessors by virtue of its ability to allosterically activate nicotinic receptors (Corey-Bloom, 2003). Although, there continues to be debate as to the extent to which nicotinic receptor activation plays a role in the drug's profile of therapeutic benefit, the concept supports the continued development of bi-functional (AChE/nicotinic) drugs. There is no question that the development of multi-functional drugs presents additional problems for rationale drug design methodologies. For example, it may prove difficult to insure that the different components of the desired responses occur at similar levels of affinities for their respective targets. This situation may be the case as discussed above for galantamine in which the drug's affinity for the nicotinic site may be too low for practical dosing regimens. There also exists the potential problem of selecting structural characteristics when screen large chemical libraries. Yet from our studies over the past decade in which we examined a wide variety of classes of pharmacological agents in basically the same non-human primate model it seems probable that drug that target single functional components of cognition and memory will be limited in efficacy (Buccafusco and Terry, 2000). Moreover, it is likely that syndromes such as AD will require multiple drug therapy to address the varied pathological aspects of the disease. Even if the strategy of combining drugs with different therapeutic targets is workable, the development of multi-functional compounds will obviate the challenge of administering multiple single drug entities with potentially different degrees of bioavailability, pharmacokinetics, and metabolism. Also, the simplification of the therapeutic regimen for individuals with AD who have difficulty with compliance is important.

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