Allosteric Sensitization of Nicotinic Receptors by Galantamine, a New Treatment Strategy for Alzheimer’s Disease

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Cholinesterase inhibitors are the only approved drug treatment for patients with mild to moderately severe Alzheimer’s disease. Interestingly, the clinical potency of these drugs does not correlate well with their activity as cholinesterase inhibitors, nor is their action as short lived as would be expected from purely symptomatic treatment. A few cholinesterase inhibitors, including galantamine, produce beneficial effects even after drug treatment has been terminated. These effects assume modes of action other than mere esterase inhibition and are capable of inducing systemic changes. We have recently discovered a mechanism that could account, at least in part, for the above-mentioned unexpected properties of some cholinesterase inhibitors. We have found that a subgroup of cholinesterase inhibitors, including galantamine but excluding tacrine, directly interacts with nicotinic acetylcholine receptors. These compounds, named allosterically potentiating ligands, sensitize nicotinic receptors by increasing the probability of channel opening induced by acetylcholine and nicotinic agonists and by slowing down receptor desensitization. The allosterically potentiating ligand action, which is not necessarily associated with cholinesterase inhibition, has been demonstrated by whole-cell patch-clamp recordings to occur in natural murine and human neurons and in murine and human cell lines expressing various subtypes of neuronal nicotinic acetylcholine receptors. Biol Psychiatry 2001;49:279–288 © 2001 Society of Biological Psychiatry

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Introduction

Alzheimer’s disease (AD) is accompanied by several distinct cellular and molecular events including the accumulation and deposition of β-amyloid, the hyperphosphorylation of Tau protein and formation of neurofibrillary tangles, the reduced expression of nicotinic acetylcholine receptors (nAChRs) and other cholinergic markers, and the presence of particular risk factor genes and mutations. It still remains an open question whether these events are linked to each other or occur independently, and whether one of them represents the primary cause of the disease. Recent genetic studies have not helped to clarify this question, but rather point to AD as a disease with many, possibly independent, risk factors. As a case in point, although the apolipoprotein e4 allele is commonly considered the most well-established risk factor gene in AD, the possession of the e4 allele is neither necessary nor sufficient to cause the disease. Accordingly, the allele is a factor that may well interact with other genetic, cellular, or environmental risk factors, before or during the development of the disease.

It still is quite common that the therapeutic benefits produced by cholinesterase inhibitor (ChEI) treatment are discounted as purely symptomatic and are hence considered not to be a complete answer for the treatment or prevention of AD. Interestingly though, most of these drugs show a delayed onset of beneficial action, and a few of them show prolonged benefits in terms of global function, cognition, and activities of daily living, even after treatment has been terminated. These effects are inconsistent with a purely symptomatic action, but rather suggest changes in molecular and cellular expression that directly affect the pathomechanism of the disease.

In this review we summarize recent findings from this and other laboratories pointing to a central role of nAChRs in AD, and present and discuss a novel approach to AD treatment based on drugs that upregulate the activity of selected subtypes of nicotinic receptors. We have identified galantamine (Reminyl) as a prototypic compound of...
this novel class of nAChR ligands. The mechanism of action of these drugs may account for not only short-lived symptomatic effects, but also long-lasting metabotropic and cellular responses, as have been noticed in clinical phase III studies.

**Nicotinic Receptors in AD**

From the observation that the muscarinic antagonist scopolamine produced deficits in short-term memory, it was initially proposed that the cholinergic deficit in AD is predominantly of a muscarinic nature (Davis and Yamamura 1978; Levey 1996). This view, however, has been challenged by a large body of evidence, including autoradiographic and histochemical studies of autopsy brain tissue (Nordberg and Winblad 1986; Perry et al 1995; Schröder et al 1991; Whitehouse et al 1986) and brain imaging studies of AD patients (Nordberg et al 1995), that points to a specific loss in nicotinic rather than muscarinic acetylcholine receptors. In contrast, these data consistently show that muscarinic receptors, including M2 receptors, are much less, if at all, reduced in AD. In the upper cortical layers of the frontal cortex and in the temporal cortex the loss of nAChRs appears to predominantly affect the α4 subunit–bearing subtype, rather than the α7 nAChR, as has been established by histochemical studies (Martin-Ruiz et al 1999) and radioligand binding (Potter et al 1999; Warpman and Nordberg 1995).

**Properties of Neuronal Nicotinic Receptors**

Neuronal nAChRs belong to the superfamily of transmitter-gated ion channel proteins. Like their muscle and electric fish counterparts, they are believed to be of pentameric structure, with all five subunits contributing to the integral cation channel (Bertrand and Changeux 1995; Lindstrom 1997). The α subunits, of which eight have been identified by cloning, contain the major parts of the binding sites for acetylcholine and competitive and non-competitive ligands, whereas the β subunits, of which three have been identified by cloning, are structural. Most β neuronal nAChRs are composed of α and β subunits, with the exception of the α7, α8, and α9 nAChRs, which are probably homopentameric. Depending on their subunit compositions, the neuronal nAChRs differ in their ion permeabilities and kinetics of channel opening and closing, and in their pharmacology. Of the many nAChR subtypes that are expressed in the mammalian brain, α4β2 [meaning that the nAChR is composed of α4 and β2 subunits, with a putative stoichiometry of (α4)2(β2)3] and α7 [(α7)5] subtypes are the most prominent ones found in both postsynaptic and presynaptic, perisynaptic, and extrasynaptic locations (Albuquerque et al 1996, 2000; Lindstrom 1997). The α7 nAChR displays functional properties quite different from those of the α4β2 nAChR, with a much higher Ca2+ permeability, very fast desensitization, and different pharmacology, including activation by choline and blockade by the elapid snake neurotoxin α-bungarotoxin (Alkondon et al 1999; Castro and Albuquerque 1995). Due to its sensitivity to choline, the α7 nAChR can be chemically excited even after the natural transmitter has been enzymatically cleaved. Other nAChR subtypes are much less, if at all, sensitive to choline, which agrees with the classical view of acetylcholinesterase (AChE) as initiator of termination of cholinergic excitation by acetylcholine cleavage. The α7 nAChR, therefore, can respond not only to synaptic events of acetylcholine release, but also to volume changes in acetylcholine/choline concentration. (Rapid desensitization of the α7 nAChR and a significant refractory period may be prerequisites for the latter response mode.) Due to its Ca2+ permeability, α7 nAChR activation can produce metabotropic responses in the excited cell, including Ca2+-controlled transmitter release (Alkondon et al 2000a) and stimulation of gene transcription and protein biosynthesis. Very recently, the first electrophysiologic studies of human cerebral cortical interneurons have been reported (Alkondon et al 2000b). These studies established that both α4β2 and α7 nAChRs are located on the somatodendritic regions of human interneurons and, as demonstrated by their ability to modulate γ-aminobutyric acid (GABA) release, could be involved in inhibitory and disinhibitory mechanisms in the human cortex. The inhibitory action could enhance the signal-to-noise ratio of neuronal circuitry, whereas the disinhibitory action could lead to synaptic strengthening, which is an essential element of the learning paradigm long-term potentiation (Alkondon et al 2000a, 2000b). So far, such properties of acetylcholine action in the human cortex have all been attributed to muscarinic receptors (Raiteri et al 1990; Russo et al 1993). Together with the receptor expression data discussed above and the fact that clinical studies with muscarinic agonists have not been able to demonstrate significant beneficial effects in AD, it therefore seems reasonable to focus the original “cholinergic hypothesis” of Bartus et al (1982) on nicotinic cholinergic neurotransmission.

There is a large body of evidence indicating that nicotinic drugs indeed affect learning and memory. Nicotine and other nicotinic agonists can improve cognitive and psychomotor function (Wilson et al 1995; Wonnacott et al 1999), whereas nicotinic antagonists lead to cognitive impairment (Newhouse et al 1988, 1994). Moreover, the incidence of AD in smokers is lower than that in non-smokers (Nitta et al 1994), which may relate to the increased nAChR expression levels observed in the brains of smokers (Nordberg et al 1995; Perry et al 1999). Thus,
nicotinic drugs may have both acute and chronic effects on cognitive function, the chronic effects possibly including a neuroprotective effect due to the upregulation of expression of α7 and α3 subunit–bearing subtypes (Marks et al 1992; Peng et al 1997).

Nicotinic receptors are also associated with anxiety and depression, which are established symptoms in AD. Nicotine is known to have anxiolytic properties; therefore, people with depression are more likely to smoke than those who do not (Covey et al 1998). Moreover, nicotine is known to mediate addiction and tolerance in chronic tobacco users (Benowitz 1996).

Neuronal nicotinic receptors have been associated with several additional psychiatric diseases, including schizophrenia (Freedman et al 1997), autosomal dominant frontal lobe epilepsy (Steinlein et al 1995), Parkinson’s disease (Whitehouse et al 1983), and Tourette’s syndrome (Dursun and Reveley 1997; Silver et al 1995). The overlapping pathology of these diseases may be due to presynaptic nicotinic receptors modulating the release of transmitters other than acetylcholine (Albuquerque et al 1996; Alkondon et al 1999).

**Nicotinic Receptors Modulate Other Neurotransmitter Systems**

The key feature of AD is a loss in cognitive function, which includes loss of (short-term) memory and learning ability, impaired attention associated with relentlessness, disturbances of language, and emotional instability. All of these functional deficits are the result of impaired neurotransmission in the central nervous system and probably involve several transmitter systems. Of the neuropeptides expressed in the human brain, however, a substantial loss in the brain regions known to be essential for the behavioral tasks that are impaired in AD has only been observed for nicotinic receptors (Martin-Ruiz et al 1999; Nordberg et al 1989; Whitehouse et al 1986). The question therefore arises whether a link can be found between nicotinic cholinergic activity and other neurotransmitter systems, so that impaired nicotinic transmission would in turn impair other neurotransmission systems.

One such link has recently been identified—namely, the modulatory control of transmitter release by presynaptic nicotinic receptors, both the α4β2 subtype and the Ca2+-conducting α7 nAChR subtype (Albuquerque et al 1996; Alkondon et al 1999, 2000a). Thus, in the case of AD, reduced expression of presynaptic nAChRs could limit or even abolish the modulatory control of glutamate release, which in turn could lead to reduced capability in learning and memory. Moreover, because α7 nAChRs can be activated by choline (Alkondon et al 1997) and not only by acetylcholine, as with most other nicotinic receptors, choline may act as a “messenger” (Maelicke et al 1995) in the learning paradigm long-term potentiation, which is governed by glutamatergic neurotransmission. Similarly, a loss of presynaptic nAChRs could also reduce the modulatory control of serotonergic neurotransmission, leading to the mood changes known to be associated with AD. The recent suggestion of an ambient level of acetylcholine in the central nervous system (Descarries 1998) is further evidence for acetylcholine/choline-controlled modulatory mechanisms. An additional mechanism of modulatory control by nicotinic receptors is provided by the considerable Ca2+ permeability of some subtypes, which links nicotinic neurotransmission to intracellular signalling controlled by Ca2+.

**Nicotinic Agonists and ChEIs as a Drug Treatment Strategy in AD**

Several nicotinic agonists are presently in preclinical and clinical testing (Dursun and Reveley 1997; Francis et al 1999; Maggio et al 1998; Newhouse et al 1997; Sabbagh et al 1998) despite the fact that they are difficult to dose, since higher doses may cause desensitization rather than increased activation of nicotinic receptors (Maelicke and Albuquerque 1996). To cope with these drawbacks, low-affinity nicotinic agonists such as ABT-418 have been developed. When they administered ABT-418 compound at three relatively low doses, conductors of a small placebo-controlled study reported a significant dose-related improvement in several areas of cognition and behavior within a few hours of administration that lasted a couple of hours thereafter (Potter et al 1999). These and other studies indicate that nicotinic cholinergic therapy seems feasible, producing at least the expected symptomatic effects.

Treatment with ChEIs may also be considered as a means of administering a nicotinic agonist, since inhibition of the acetylcholine-degrading enzymes acetylcholinesterase and butyrylcholinesterase increases the synaptic level of the natural agonist acetylcholine. The risk associated with the overdosing of ChEIs is a well-established clinical fact, documented by many incidences of carbamate and organophosphate poisoning, including purposeful application of these compounds as insecticides and poison gas. As a consequence, most therapeutically applied ChEIs are of low potency and belong to the groups of reversible inhibitors (e.g., tacrine and galantamine) or groups of slowly reversible covalent inhibitors (e.g., physostigmine and rivastigmine) of these serine hydrolases. In the application of ChEI as drugs, further matters of concern are side-effects produced by muscarinic overstimulation and the recent finding that various cholinesterase isoforms exist in the human brain, with some of them...
possibly representing novel risk factors in neurodegenerative diseases such as AD (Kaufer et al. 1998; Soreq et al. 2000). Taken together, therapeutic approaches using nicotinic agonists or ChEIs require low-affinity nAChR subtype- and cholinesterase isoform-specific compounds so as to reduce the risk of adverse side effects.

Positive Allosteric Modulation of Human Nicotinic Receptors by Galantamine

So far, the most successful psychiatric drugs have not been receptor agonists, but rather compounds that act in an allosteric fashion on these targets. Arguably the most prominent example is the benzodiazepines, which positively modulate (sensitize) the GABA<sub>A</sub> receptor by facilitating a GABA-induced opening of the receptor-integral Cl<sup>-</sup> channel (increase in the probability of channel opening at given concentrations of GABA). This effect is the underlying principle of the anxiolytic activity of benzodiazepines (McDonald and Twyman 1992). Similarly, it should be very useful to have nicotinic drugs that positively modulate (sensitize) selected subtypes of neuronal nAChRs in a benzodiazepinelike fashion. We have indeed succeeded in identifying such compounds (Schrattenholz et al. 1996), with the surprising finding that some of these nAChR-sensitizing agents were established AChEIs, such as the AD drugs galantamine and physostigmine. Therefore, these compounds have a dual mode of action.

The nAChR-sensitizing agents identified by us (Maelicke and Albuquerque 1996; Maelicke et al. 1995; Schrattenholz et al. 1996) all appear to interact with the receptor via binding sites that are distinct from those for ACh and nicotinic agonists and antagonists. Consequently, we named them allosterically potentiating ligands (APLs). As a particular advantage, APLs are not directly involved in the neurotransmission process they affect. Hence, they usually do not induce compensatory processes, as agonists...
and antagonists may do (e.g., receptor desensitization, downregulation of expression).

A representative example of allosteric potentiation of nicotinic responses is shown in Figure 1A. Using the cell line Hα4β2L/1, which is the human embryonic kidney cell line HEK-293 stably transfected with the human α4β2 neuronal nAChR, the response to 100 μmol/L acetylcholine in the absence of galantamine (first trace) was significantly increased in peak amplitude when acetylcholine was applied together with 0.5 μmol/L galantamine (third trace). At the same concentration, galantamine alone did not induce a significant whole-cell current (second trace). After removal of the drugs, reapplication of acetylcholine (100 μmol/L) produced approximately the same response as originally observed in the absence of galantamine (fourth trace).

Previous studies with several other cell lines (Pereira et al 1994; Schrattenholz et al 1996; A. Maelicke et al, unpublished data) and cultured hippocampal neurons (Pereira et al 1993) have demonstrated that the potentiating effect of galantamine can be inhibited by the monoclonal antibody FK1, which specifically blocks the galantamine-binding site without interfering with the acetylcholine binding site (Schröder et al 1994). Accordingly, the action of galantamine and structurally related drugs is allosteric, rather than via the acetylcholine-binding sites.

A weak agonist action of the acetylcholinesterase inhibitor physostigmine was first described by Katz and Miledi (1977) and later by Shaw et al (1985). Since it has been demonstrated that this action is actually an allosteric potentiation of an intrinsic ACh response (Schrattenholz et al 1996), several other laboratories have reported similar findings (Buisson and Bertrand 1998; Krause et al 1998; Sabey et al 1999; Zwart and Vijverberg 1997). Thus, whereas the existence of positive allosteric modulation of nicotinic receptors has been established by several independent laboratories, there still remains some disagreement as to the underlying molecular mechanism (Zwart et al 2000).

The effect of galantamine on the dose–response relationship for acetylcholine is shown in Figure 1B. The APL shifted the dose–response curve to lower concentrations of acetylcholine without significantly changing the level of maximal response (not shown). This finding suggests that, in the presence of galantamine, the affinity of binding of acetylcholine to the α4β2 nAChR is increased. Furthermore, galantamine increases the steepness of the dose–response curve for acetylcholine, concomitantly increasing the Hill coefficient (from 1.1 to 1.6), which suggests an improvement in the allosteric interaction between the acetylcholine binding site–bearing α4 subunits (of which there are at least two in functional nAChRs).

The potentiating effect of galantamine is observed only in a rather narrow window of APL concentration. As shown in Figure 1C, potentiation of acetylcholine-induced response was limited to galantamine concentrations below 5 μmol/L. At higher concentrations of galantamine, direct channel blockade is becoming increasingly significant; it counterbalances and eventually reverses the potentiating effect.

Similar studies using HEK-293 cell lines that stably express other human nAChR subtypes are in progress in our laboratory (Samochcki et al, in press). In the case of the homomeric α7 nAChR subtype, we produced several T-Rex HEK-293 cell clones stably expressing a chimeric receptor, which was composed of the N-terminal extracellular region from the chicken α7 nAChR (amino acids 1-201) and the remaining transmembrane and other regions from the serotonin (5-HT3 ) receptor (Eisele et al 1993). Because the chimeric receptor is a functional cation channel that largely displays the pharmacologic profile of the α7 nAChR (Samochcki et al, in press), it can be applied to testing whether ACHEIs such as galantamine also act as APLs on the α7 subtype. Figure 2 demonstrates dose–response curves for acetylcholine, in the absence and presence of 0.5 μmol/L galantamine, acting on the cell line Ca7/5-HT3/L/1. These data indicate that the α7 nAChR is indeed subject to allosteric sensitization by
APLs, and that galantamine seems to produce an even larger increase in acetylcholine response amplitude than in the case of the α4β2 subtype (Figure 1B). Ivermectin has been previously identified as a positive allosteric effector of the α7 nAChR expressed in Xenopus oocytes and K-28 cells (Krause et al 1998). As a preliminary generalized conclusion, all nAChR subtypes tested so far have displayed an APL effect. This is in agreement with the results from binding site and epitope mapping studies, indicating that the sequence region in which major elements of the APL binding site are located is highly conserved between nAChR α subunits (Schrattenholz et al 1993; Schröder et al 1994).

**Structural Requirements and Underlying Mechanism of APL Action**

Representative nicotinic APLs are the plant alkaloids physostigmine, galantamine, and codeine and the neurotransmitter 5-HT. Most APLs are rather lipophilic compounds, and they contain a tertiary nitrogen that is cationic at neutral pH and located at a fixed distance from a phenolic hydroxyl group (Maelicke et al 1995). These structural properties are similar to those of phanenothrene-type opioids and endorphins with narcotic activity. They are also found in nonnarcotic drugs, such as certain dopaminergic agonists and antagonists, and some centrally acting cholinergic drugs. The structural properties of APLs are quite different from those of classic nicotinic agonists and antagonists (Maelicke 1984). This agrees with the results of photoaffinity labeling studies using Torpedo membrane fragments as nAChR preparation and physostigmine as photactivatable ligand, which showed that the binding site for APLs is located around and including Lys-125 of the N-terminal extracellular region of the α subunit (Schrattenholz et al 1993). Although located in the same subunit, the APL binding region is distinct from that described for acetylcholine binding (Bertrand and Changeux 1995). Moreover, a monoclonal antibody raised against the APL-binding region (mAb FK1) blocks binding of APL to Torpedo nAChRs, without interfering with acetylcholine binding and with binding of an mAb raised against the acetylcholine site (mAb WF6) (Schröder et al 1994).

The structural properties of APLs are also different from those AChEIs. Thus, the APL codeine does not interact with the enzyme. The APL galantamine is a rather weak AChEI (Kᵢ = 0.14 μmol/L), and in the covalent AChEI physostigmine, the carbamate function can be removed without loss in potency as an APL, whereas the same treatment reduces the potency of physostigmine as an AChEI by several orders of magnitude. Moreover, many well-established AChEIs, including tacrine (Figure 1C) and metrifonate, do not act as APLs on α4β2 nAChRs (Samochocki et al, 2000). With regard to tacrine, our findings are in disagreement with the results of Svensson and Nordberg (1996), who reported an APL action on α4β2 nAChRs expressed in M10 cells. Because many of the centrally acting ChEIs and APLs are rather hydrophobic and do not have a fixed positive charge, they tend to partition into cell membranes to an extent that can affect electrophysiologic recordings, and biochemical binding and transmitter release studies. This may result in response enhancements that typically are poorly concentration dependent, if at all, and do not reproduce well. Studies with tacrine are particularly prone to such artifacts. Another misunderstanding of the mechanism of action of APLs may result from the fact that the potentiating effect of APLs is limited to submaximal nicotinic responses. This has been misinterpreted by others (Zwart et al 2000) as representing competition for the same binding sites on the receptor, even though APLs do not produce a significant whole-cell response on their own, and thus additivity of response (of acetylcholine and APLs) is not observed at reasonable concentration. Moreover, these authors did not make use of site-specific inhibitors, such as the antibody FK1, and they also did not identify the binding sites for the ligands used in their studies by affinity labeling.

Further evidence on the mode of action of APLs is provided by patch-clamp single-channel recordings. Although APLs do not induce any significant macroscopic currents (Figure 1A), galantamine and related compounds can induce single-channel activity in excised patches from various cells, including cultured rat hippocampal neurons (Pereira et al 1993), M10 cells (Pereira et al 1994), and PC12 cells (Storch et al 1995), with ion-channel conductances that are indistinguishable from those induced by acetylcholine. The single-channel activity could not be blocked by established nicotinic antagonists, demonstrating again that it was induced via a site separate from that for acetylcholine and competitive ligands. Further electrophysiologic studies established that APLs generally have an efficacy that is too low as agonist to produce significant whole-cell currents. To add to the low probability of channel opening, APLs are powerful direct blockers of the nAChR channel (Pereira et al 1993; Schrattenholz et al 1996). The single-channel activity induced by APLs identifies them as “noncompetitive agonists” of very low efficacy (Maelicke et al 1995; Storch et al 1995). Thus, APLs are capable of inducing conformational changes in the nAChR that are in the direction of the open channel conformation; however, this state is not achieved with significant probability. In contrast, when applied together with acetylcholine or an agonist, APLs can cooperate with the agonist in moving the nAChR to the open-channel state. The result is a facilitation of channel activation by the agonist, which on the single-channel level is seen as an increase in the frequency of channel opening (Pereira et al 1996).
On the macroscopic level, APLs increase the number of open states (increase in response amplitude), which, in the framework of the allosteric model, could be interpreted as an increase in binding affinity for acetylcholine (Figure 1) (Maelicke et al 1995). Nicotinic APL as Drug Candidates in AD Figure 3 summarizes the reaction pathway of acetylcholine from release to degradation. After presynaptic release, acetylcholine interacts with the hydrolyzing enzyme acetylcholinesterase in the synaptic cleft and with nAChRs in the subsynaptic membrane. Because the association of acetylcholine with both macromolecules proceeds with a very high, almost identical rate (Jurss et al 1979), the relative partition of acetylcholine is determined by the expression levels and accessibility of acetylcholinesterase and nAChRs. Eventually all acetylcholine molecules will be degraded to choline and acetate, and the breakdown products will be removed by diffusion and reuptake. In this sense, interaction of acetylcholine with nAChRs is a delay loop in the pathway from acetylcholine release via enzymatic inactivation to removal (Maelicke 1984).

Drugs can affect nicotinic neurotransmission in three ways: 1) by inhibition of acetylcholinesterase, thereby temporarily raising the synaptic level of acetylcholine and hence the probability of nAChR activation; 2) by sensitization of nAChRs (e.g., by APLs); and 3) by increasing the stability of the open-channel state (slowing-down of channel-closing rate. Most drugs presently used in AD act only as acetylcholinesterase, whereas galantamine and physostigmine also act as nAChR-sensitizing ligands (APLs). Codeine does not interact with acetylcholinesterase, but acts as an APL on nAChRs. Weighting possible strategies for drug treatment in AD, obviously the most specific one and hence the least prone to unwanted side effects is to selectively sensitize those nAChR subtypes that are reduced in expression in AD (Martin-Ruiz et al 1999). Presently, the most common approach (i.e., inhibition of acetylcholinesterase) is certainly more likely to produce side effects, including those associated with muscarinic overstimulation. An interesting alternative is drugs with a dual action (i.e., that act as both AChEI and APLs) (Maelicke and Albuquerque 1996, 2000; Schrattenholz et al 1996). One such drug is galantamine (Reminyl), which has already undergone phase III clinical studies with very promising results, including a sustained clinical benefit. According to our studies, galantamine is quite a powerful APL, but a rather weak reversible AChEI. Galantamine’s therapeutic value may therefore mainly result from its APL activity. In contrast, physostigmine is a weaker APL but a stronger AChEI. Moreover, unlike galantamine, physostigmine has a short half-life and poor tolerability, which limit its clinical use (Nordberg and Svensson 1998; Thal et al 1999).

In addition to the apparently immediate (symptomatic) effects of ChEIs and APLs that are addressed in Figure 3, the clinical studies with galantamine indicated a long-lasting action and a slowing down of disease progression. These stabilizing effects are unlikely to be due to acetylcholinesterase inhibition and/or increased depolarization mediated by nAChRs. Instead, they could result from metabotropic responses associated with nicotinic stimulation. There have been initial reports suggesting links between some ChEIs and nicotinic agonists, their target proteins and β-amyloid metabolism (Kihara et al 1998; Lahiri et al 2000) and aggregation (Wang et al 2000; Inestrosa and De Ferrari 2000), nicotinic receptor and nerve growth factor biosynthesis, and other neuroprotective effects. It has therefore been suggested that the relatively high Ca$^{2+}$ permeability of the α7 nAChR subtype (Albuquerque et al 1996) with this second messenger is capable of inducing a plethora of metabotropic responses, including gene regulation.

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