New approach to drug therapy in Alzheimer's dementia

Alfred Maelicke and Edson X. Albuquerque

Most current drug therapeutic approaches to Alzheimer's dementia are aimed either at elevation of the transient levels of acetylcholine in the brain or at direct enhancement of nicotinic receptor activation by the application of cholinesterase inhibitors or nicotinic agonists, respectively. One problem of the latter approach is agonist-induced desensitization of nicotinic receptors. An alternative option, which could circumvent this problem, involves a novel class of nicotinic ligands, which potentiate the response of nicotinic receptors to acetylcholine by acting from an allosteric site. The mechanism of action and the structure–function relationship of these potential drugs is discussed.

Alzheimer's dementia (AD) is a neurodegenerative disorder of the CNS that is characterized by profound memory impairment, emotional disturbance and, in late stages, by personality changes. The disease usually occurs in elderly people, but inherited forms have been reported in patients as young as 40 years. The incidence of AD in elderly people is age-dependent, increasing from less than 1% at 60–65 years of age to more than 30% [possibly as high as 47% (Ref. 1)] after the age of 85 years.

Studies of brain tissue from autopsy indicate that AD is accompanied by neuronal loss, synaptic damage, and increased levels of neurofibrillary tangles, neuritic plaques and granulovacuolar degeneration[2,3]. At the molecular level, the major components of the tangles and plaques have been identified, and it has been found that AD is associated with reduced levels of choline acetyltransferase (ChAT), acetylcholinesterase (AChE) and nicotinic acetylcholine receptors (nAChR)[4-6].

Etiology and the 'cholinergic hypothesis'

Several causes of AD have been proposed, including the following:

- Extracellular depositions of amyloid protein (β-AP), resulting from overexpression and/or incorrect metabolism and transport of the amyloid precursor protein (APP), and of amyloid-associated proteins[7]
- Hyperphosphorylation of tau proteins[8]
- Glutamate-induced acute neurodegeneration[9]
- Oxidative damage
- Apoptotic cell death
- Cholinergic dysfunction[6,10]

Of the putative causes, the 'cholinergic hypothesis'[10] offers the best prospect for rational drug design because the cholinergic dysfunction is intimately associated with the symptoms of AD, and an observed reduction in the concentration of nAChRs correlates with the severity of the symptoms[6,10,11]. In contrast, the concentrations of other neuroreceptors, including muscarinic acetylcholine receptors (mAChR), glutamate and 5-hydroxytryptamine (5-HT) receptor subtypes, are comparable with levels in age-matched controls[6,11–13]. These findings suggest that general neurodegeneration is not the major cause of AD. Reduced levels of nicotinic receptors in AD have also been shown by

Alfred Maelicke*, Laboratory of Molecular Neurobiology, Institute of Physiological Chemistry and Pathobiochemistry, Johannes–Gutenberg University Medical School, 55099 Mainz, Germany. **Edson X. Albuquerque**, Department of Pharmacology and Experimental Therapeutics, University of Maryland Medical School, Baltimore, MD 21201, USA, and Laboratorio de Farmacologia Molecular II, Instituto de Biofisica 'Carlos Chagas Filho', Universidade Federal do Rio de Janeiro, RJ 21944, Brazil. *tel: +49 6131 395911, fax: +49 6131 393536, e-mail: alfred.maelicke@uni-mainz.de

  1359-6446/96/$15.00

postmortem autoradiographic binding studies with [^{3}H] nicotine and by brain imaging PET studies with [^{11}C] nicotine[14,15].

There is much evidence to indicate that neuronal nicotinic receptors play an important role in learning and memory[16], and nicotine, and other nicotinic agonists, have been reported to improve cognitive functions[17] and the performance of animals in learning paradigms[18]. This view is supported by the observation that the incidence of AD in smokers of old age is approximately 60% lower than in nonsmoking individuals[19]. In smokers, the concentration of nAChRs in the brain is significantly elevated[20], and fewer neuritic plaques and less neurodegeneration are observed[21]. When considered together with the reduced numbers of nicotinic receptors and cholinergic terminals in the brain of AD patients[6,11], these findings suggest a close relationship between nicotinic receptors and AD, in the sense that the reduced number of nAChRs could itself be the primary cause of the disease, or else might be the symptomatic consequence of another primary cause (see above). It must be emphasized that nicotinic receptors, being nonspecific cation channels, are permeable to Ca^{2+} and are often functional at membrane potentials at which other ligand- and voltage-gated ion channels are not[22]. A reduction in the number of neuronal nAChRs could, therefore, cause imbalance in the physiological levels of synaptic excitability and of intracellular Ca^{2+}, and it is feasible that such effects mediate the impairment of cognitive function and the neurodegeneration that are characteristic of AD.

The abundance, differential expression, and distinct functional properties of brain nicotinic receptor subtypes have only recently become accessible to in-depth analysis[22–24], and it is mainly for this reason that the 'cholinergic hypothesis' has long concentrated on muscarinic neurotransmission only. It is argued that reduced muscarinic stimulation in the hippocampus and the cerebral cortex is a causal factor for cognitive impairment in AD. Because the density of mAChRs is not reduced in the brains of individuals with AD[6,11–15] relative to age-matched control individuals, this hypothesis has lost support in recent years.

In spite of the existence of several theories regarding the pathogenesis of AD, the molecular causes of the condition are still unknown. It is, however, clear that the key symptoms of AD are primarily caused by cholinergic dysfunction, which thus provides the rationale for most current approaches to AD drug therapy.

The 'cholinergic hypothesis' in drug therapy

The 'cholinergic hypothesis' has served as the rationale for the development of anti-AD drugs that include anticholinesterases and muscarinic and nicotinic agonists. Alternative approaches, such as the use of neurotrophic agents, nootropics, glutamate antagonists, benzodiazepine receptor ligands and calcium antagonists, have been discussed in a recent review volume[25].

Cholinesterase inhibitors

Cholinesterase (ChE) inhibitors, such as physostigmine, galanthamine and tacrine (Figure 1) are the experimental drugs most often studied clinically in the treatment of AD. Their application is aimed at increasing the levels of ACh in patients with AD by reducing the activity of brain cholinesterases (ChE, AChE and BuChE), which inactivate ACh by cleavage. Anticholinesterases also increase the levels of other neurotransmitters[26], such as glutamate, noradrenaline, dopamine and 5-HT. This action may result from a direct (agonist-like) action of some anticholinesterases[27] on presynaptically-located nAChRs[28] which, in turn, control the release of the other transmitters. Whether the involvement of nicotinic receptors in learning and memory is mainly by presynaptic nAChRs[28], or also by postsynaptic nAChRs[29], remains unclear. Irrespective of the final outcome of this debate, the benefits of the use of anticholinesterases in the treatment of AD are equivocal – increased levels of glutamate and dopamine may cause behavioral changes and increased neurodegeneration, whereas increased levels of 5-HT and noradrenalin may be beneficial in the treatment of noncognitive behavioral abnormalities. The clinical data so far available suggest that AChE blockers provide only limited therapeutic gains[30]. Notwithstanding, tacrine and galanthamine are the only drugs to have been approved in the USA (and in several other countries) for the treatment of AD.

Nicotinic agonists

The therapeutic application of nicotinic agonists (Figure 1) has initially focused on the increase in neurotransmitter levels[31] and the reduced degeneration of cortical neurons[32] observed after administration. Nicotine is reported to improve attention and information processing in AD patients[33], and it has recently been discovered that nicotinic agonists upregulate the concentration of neuronal nAChRs, probably by reducing the rate of turnover of these receptors[34,35]. This action of nicotine has attracted much

attention, because it may be related to the lower incidence of AD in smokers[19]. Several high- and low-affinity nicotinic agonists are presently in development as AD drugs[36], even though they are known to cause central and peripheral side-effects, including hypothermia, seizures and reduction of locomotor activity, emesis and cardiovascular effects.

Muscarinic agonists

Modest improvement of cognitive function by some muscarinic agonists has been reported[37]; this is probably caused by effects other than increased transmitter release[38]. Because mAChRs regulate cellular metabolism through G proteins, it has recently been suggested that muscarinic agonists may beneficially affect the metabolism of the amyloid precursor protein (APP), the overexpression of which is a putative primary cause of AD. At present, the beneficial effects of muscarinic drugs are equivocal[39]. These compounds cause several undesirable parasympathomimetic effects and enhance gastric and intestinal secretion.

In summary, although the 'cholinergic hypothesis' has already had an immense impact on the development of therapeutic approaches[25], the complexity of CNS neurotransmission in general, and the rather ubiquitous distribution of cholinergic neurotransmission systems throughout the body, complicate the application of most drugs so far developed. Cholinesterase inhibitors presently represent the only approved drugs for AD therapy; other cholinesterase blockers and muscarinic agonists are in advanced clinical trial, but the reservations surrounding these approaches remain.

Positive allosteric modulation of nAChR activity

Our group have recently described a novel class of nAChR ligands[40,41], which act as allosterically potentiating ligands (APL) on the nicotinic responses induced by ACh and competitive agonists (Box 1). APLs also act as noncompetitive agonists (NCA) on nicotinic receptors (Box 2; Refs 42–45), but the efficacies of APLs as NCAs are too low to be of value[41–45]. Representative members of this class of ligands are the plant alkaloids physostigmine, galanthamine and codeine[45]. The potentiation by APLs of submaximal responses to ACh (Box 1) could mean that those exerting the effect without significant side-effects could be useful therapeutic agents.

Noncompetitive agonism and allosteric potentiation of nicotinic responses by APLs probably both result from conformational changes of the receptor towards the open-channel conformation. Although the probability of inducing the full open-channel conformation is too low to produce significant macroscopic currents[41,43,45], the induced conformational changes facilitate channel activation by ACh and competitive agonists, thereby producing the potentiating effect demonstrated in Box 1.

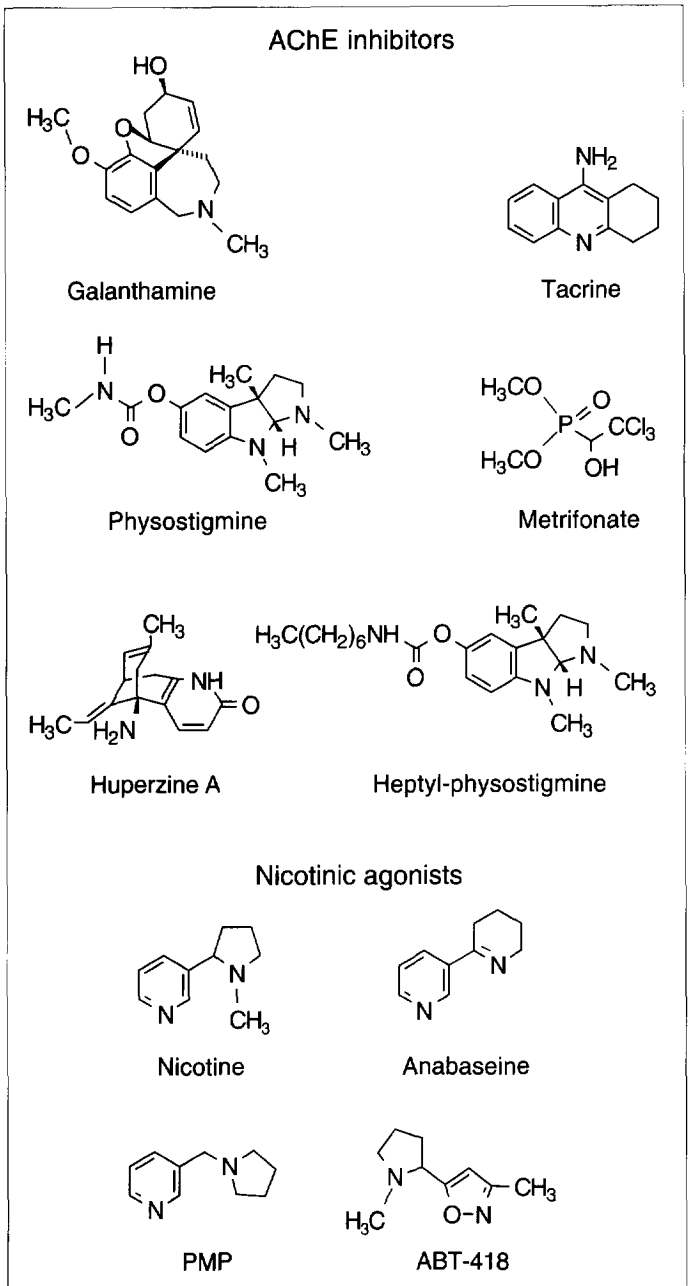
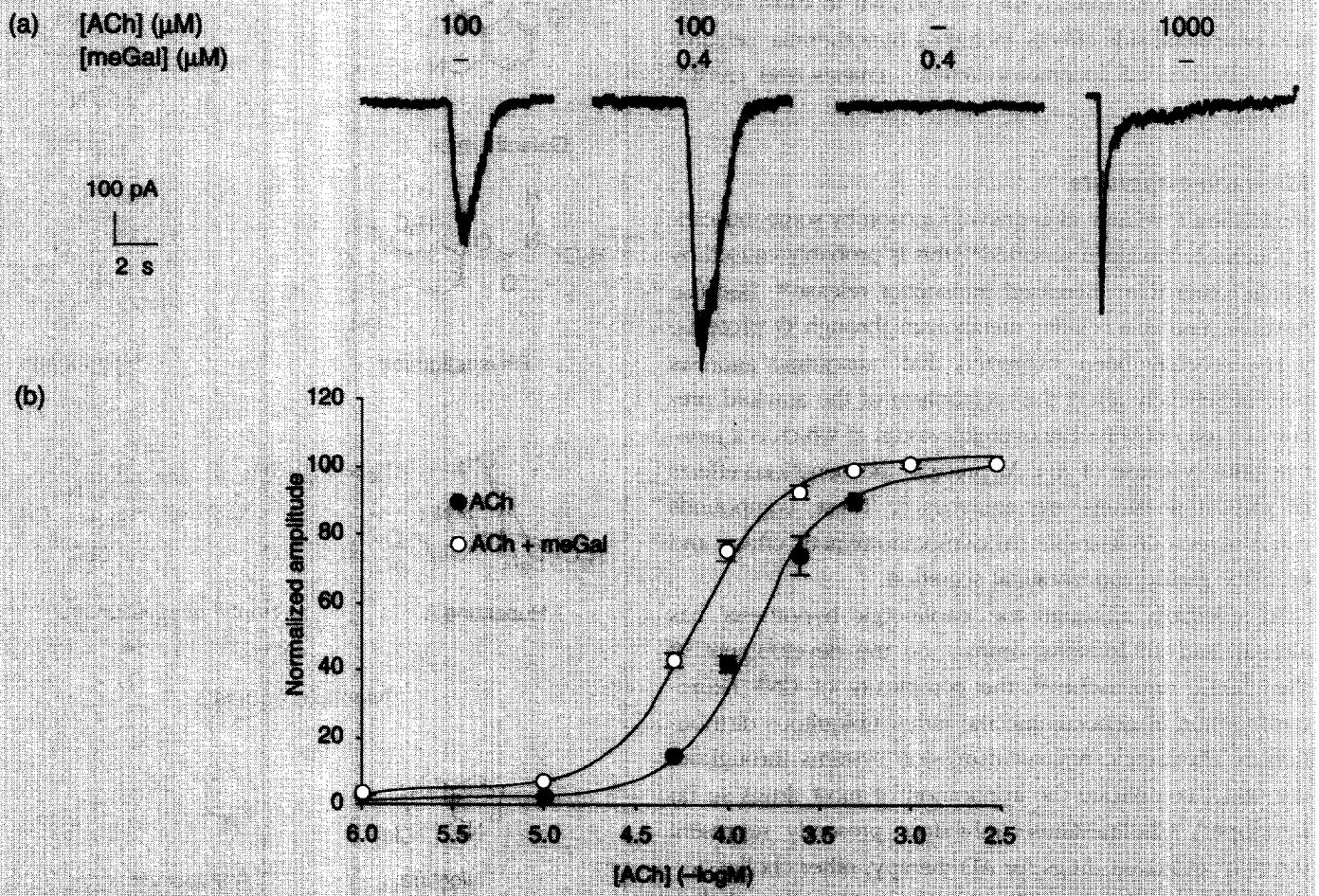


Figure 1. *Chemical structures of AChE inhibitors and nicotinic agonists under investigation as therapeutic agents in Alzheimer's dementia.*
PMP, N-(3-pyridylmethyl)pyrrolidine;
ABT-418, S-3-methyl-5-(1-methyl-2 pyrrolidinyl)isoxazole.

Box 1. Potentiation by 1-methylgalanthamine of acetylcholine-elicited whole-cell responses of cultured PC12 cells



(a) The responses were recorded from a single PC12 cell of bipolar morphology. The response to 100 μM acetylcholine (ACh), in the absence of 1-methylgalanthamine (meGal; first trace), nearly doubled in peak amplitude when the same concentration of ACh was simultaneously applied with 0.4 μM meGal (second trace). The observed potentiation of the ACh response (second trace) was not a result of mere summation of nAChR channel activity induced by ACh and the allosterically potentiating ligands (APL) because at the given concentration of meGal (and also at much higher concentrations[45]), the APL does not produce substantial single-channel activity[43,45] or whole-cell current (third trace). The potentiated response resembled in maximum amplitude, but not in the kinetics, the response to 1 mM ACh, in the absence of the APL (fourth trace). The potentiating effect on ACh responses is limited to meGal concentrations below 1 μM, as it is eventually overcome by noncompetitive inhibition of the ACh-activated channel[41]. (b) Effect of meGal (0.4 μM) on the dose–response relationship for ACh (mean of results from three experiments). The maximum amplitudes (approximately 1 nA) were normalized to 100. The presence of the APL does not affect the level of maximum response to ACh; it shifts the dose–response curve to the left. The results demonstrate that meGal, at submicromolar concentrations, potentiates submaximal ACh-induced responses of nicotinic receptors.

The positive allosteric effects of APLs on nAChR activation resemble, in some respects, the action of alcuronium on M_2 and M_4 muscarinic receptors[46]. It is particularly noteworthy that on nicotinic receptors, as on muscarinic receptors[47], the allosteric site is located in relative proximity to the orthosteric site[48]. Allosteric modulation is a widespread phenomenon in neuroreceptor regulation, the physiological role of which may be cross-talk of neighboring receptors and synapses, in the context of higher order control of CNS function[40].

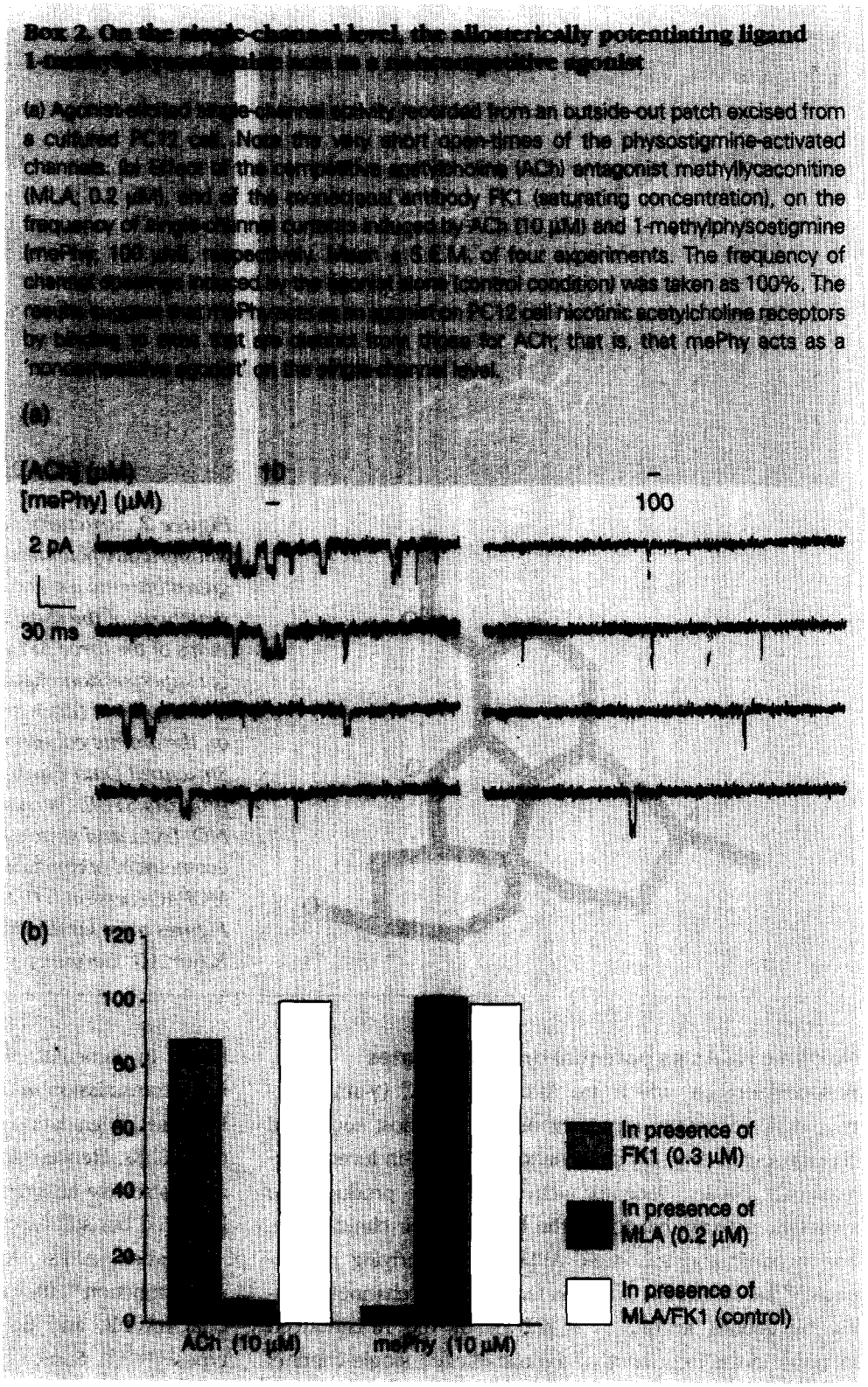
Whereas the first members of this new class of nAChR ligands were established AChE blockers[42], it soon became

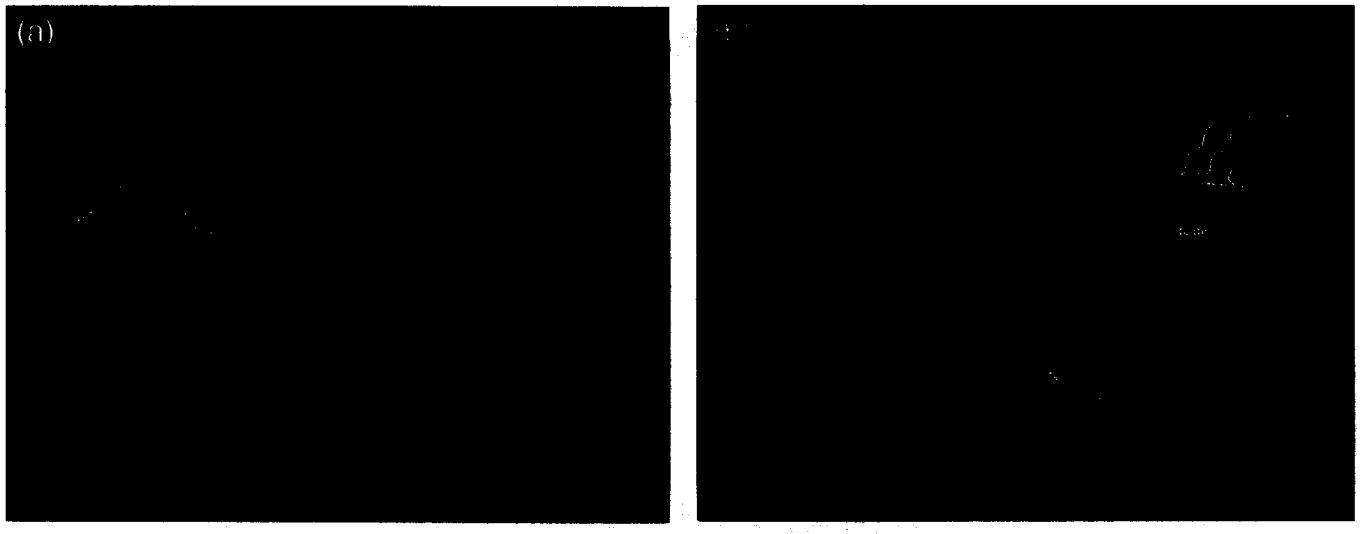
evident that neither the formation of an ester with an active serine residue nor general anticholinesterase activity was required for the drug to act as nicotinic NCA or APL (Refs 41,45). In contrast, APL activity is encoded in the structure of the aromatic moieties of these compounds[45]. APLs of the physostigmine/galanthamine/codeine subclass are relatively lipophilic compounds, with a nitrogen that is cationic at physiological pH, and which is located at a fixed distance from a phenolic hydroxyl (Figures 1 and 2). These structural properties overlap with those of some centrally-acting cholinergic drugs, some dopaminergic agonists and antagonists, phenanthrene-type opioids and general cognition enhancers[49], all of which need to cross the blood–brain barrier before interacting with the target sites in the CNS. Further elucidation of the structure–function relationships of these compounds will, therefore, remain a major goal in the development of APLs as anti-AD drugs if selectivity of action is to be optimized and side-effects minimized.

The sequence position of the APL/NCA binding site at nicotinic receptors has been identified by photoaffinity labeling with physostigmine[50], and by epitope mapping with a APL/NCA-competitive anti-nAChR antibody[48]. Besides representing an evolutionary conserved region in nAChR α-subunits, the APL site is lined by several hydrophobic residues[48]. The great similarity of this structure to that of the active site gorge of AChE (Ref. 51) may explain the anticholinesterase activity of some APLs. At elevated concentrations, the potentiating action of APLs is overcome by their direct blockade of the nAChR channel[41], which resembles the action of local anesthetic agents. This property reduces their application as allosterically potentiating ligands to a window of concentrations, the upper limit of which is about 1 μM.

Box 2. On the single-channel level, the allosterically potentiating ligand 1-methylphysostigmine acts as a non-competitive agonist

(a) Agonist-elicited single-channel activity recorded from an outside-out patch excised from a cultured PC12 cell. Note the very short open-times of the physostigmine-activated channels. In order to the competitive acetylcholine (ACh) antagonist methyllycaconitine (MLA; 0.2 μM), and of the monoclonal antibody FK1 (saturating concentration), on the frequency of single-channel currents induced by ACh (10 μM) and 1-methylphysostigmine (mePhy; 100 μM), respectively. Mean ± S.E.M. of four experiments. The frequency of channel openings (induced by the agonist alone (control condition) was taken as 100%. The results indicate that mePhy acts on PC12 cell nicotinic acetylcholine receptors by binding to sites that are distinct from those for ACh; that is, that mePhy acts as a 'non-competitive agonist' on the single-channel level.





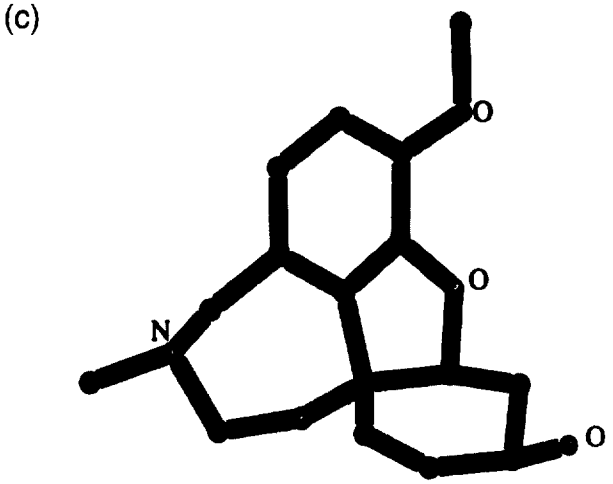


Figure 2. *Structural comparison of the nicotinic allosterically potentiating ligands (APLs) physostigmine, galanthamine and codeine. (a) Projections of the crystal structures of the three APLs; (b) electrostatic potential maps of the three APLs calculated* ab initio*; (c) superposition of galanthamine (light atoms) and* N*-norcodeine (dark atoms). Molecular models are based on the atomic coordinates stored in the Cambridge Structural Data Bank. Modeling was performed with the program SYBIL (version 6.03, Tripos Associates, St Louis, MO, USA) and structural refinement was achieved by geometrical optimization using the program package MOPAC (version 6.0, J.J.P. Stewart, QQCPE No. 455). Figures were kindly provided by Dr Oliver Gutbrod, Bayer AG, Germany.*

Nicotinic NCAs as potential drug candidates

Benzodiazepines, which are APLs of $GABA_A$ (γ-aminobutyric acid) receptors, represent one of the most successful classes of CNS drug. By potentiation of GABA-induced (submaximal) $GABA_A$ receptor activation, they produce the anxiolytic activity that forms the basis of their clinical role. For nicotinic and gabaergic APLs, the underlying mechanism of the sensitizing action is facilitation of channel opening by agonist action, as is shown by the APL-induced increase in the frequency of agonist-elicited single-channel activity[41]. Because of the similarities to the benzodiazepines, nicotinic APLs may have several advantageous properties worthy of exploration in the context of AD therapy. Because APLs act at the level of allosteric modulation, their action would be moderate, enabling careful adjustment of impaired nicotinic neurotransmission in AD to beneficial levels. In particular, nicotinic APLs could enhance nicotinic neurotransmission under conditions of reduced secretion or increased degradation of ACh, or of reduced ACh-sensitivity of nAChRs. Hence, nicotinic NCAs could have a preventive and corrective action on nicotinic neurotransmission that is impaired but still functioning.

Because APLs do not necessarily interfere with AChE function[45], they could be specifically targeted at neuronal nAChR, and possibly even at selected nAChR subtypes[22,23,52,53]. Similarly, although some nicotinic APLs with anticholinesterase activity have been shown to act directly on muscarinic AChRs[29,54], this property may not extend to APLs that do not interact with ChEs; this would further reduce the risk of side-effects.

Several nicotinic APLs that are established anticholinesterases have already shown positive results in tests

involving learning and memory tasks in animal models[55,56]. To clarify whether the new principle of allosteric potentiation of nicotinic responses also applies to *in situ* conditions of brain activity, APLs without anticholinesterase activity require investigation in such models. Two animal models of particular interest for research on AD have recently become available, one overexpressing human ChE (Ref. 57), the other human APP (Ref. 58). Of these, transgenic mice overexpressing human ChE have been shown to be impaired in cognition[57], which strengthens the conceptual correlation between cholinergic imbalance and cognitive capacity; that is, the 'cholinergic hypothesis'.

Several nicotinic APLs have already been entered into clinical trials, albeit on the basis of anticholinesterase activity[59,60]. These compounds may provide a good starting point for the development of new APLs that act exclusively on nicotinic receptors, rather than on other targets such as cholinesterases or other neuroreceptors. If these then perform successfully in animal and clinical tests, positive allosteric modulation of nicotinic receptors could become a new therapeutic concept in Alzheimer's dementia.

ACKNOWLEDGEMENTS

We thank Dr Oliver Gutbrod from Bayer AG, Leverkusen, Germany for performing the molecular modeling studies used in Figure 2. Dr Andre Schrattenholz and Dr Uli Roth provided important ideas and suggestions about this manuscript. Work from our laboratories is supported by grants from the German Science Foundation (DFG), the Fonds der Chemischen Industrie, the Volkswagen Foundation, the National Institutes of Health and from Bayer AG.

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