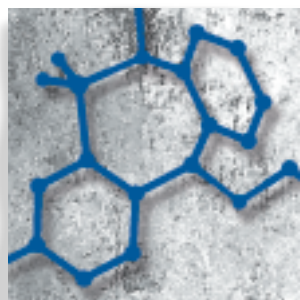


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Pharmacological models in Alzheimer's disease research

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Wider use of pharmacological models would facilitate the development of new drugs for Alzheimer's disease (AD). The two main models currently used are based on the cholinergic and glutamatergic hypotheses of AD. Although they lead to some of the attention and memory impairment observed in AD, they do not fully reproduce the AD pattern. The few studies that used a combination modeling approach, ie, the simultaneous administration of several drugs with the aim of impairing several neurotransmitters or different aspects of a single system, have reported no or marginal cumulative effect. On the basis of current understanding of glutamate and acetylcholine involvement in AD pathophysiology, we suggest that models using selective muscarinic-1 (M_1) receptor blockers would better mimic the status of the cholinergic system in AD. This kind of model might be suitable for the assessment of drugs that do not act directly on the cholinergic system.

Keywords: Alzheimer's disease; dementia; model; scopolamine; ketamine; pirenzepine; acetylcholine; glutamate

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All the professionals involved are convinced that finding effective treatments for Alzheimer's disease (AD) should be a priority for the pharmaceutical industry. AD is a wonderful challenge for industry. However, research and development in this field can also be a risky business. There is currently no consensus on the pathophysiology of AD on which drug development can rely. The clinicopathologic picture that we call AD may actually be a syndrome, with many possible causes. As a consequence, we still have no reliable, positive diagnostic test that can be applied on an individual basis, which leads to the risk of recruiting very heterogeneous patient populations for clinical trials. The low response rate to acetylcholine esterase inhibitors probably illustrates these uncertainties.

Before starting expensive trials, pharmaceutical companies clearly need to assess the validity of the underlying concept in the early phases of development. Part of the answer can come from animal models. However, if a pharmacological effect is observed, it must still be confirmed in humans.

Some advocate early administration to patients, but this is not necessarily the simplest method. The risk of heterogeneous recruitment to clinical trials is an important point. If the goal is to measure clinical improvement, the drug will probably be administered for a long period of time. If the trial intends to assess changes in surrogate markers, these must be defined. Recruiting groups homogeneous for a selected marker can be difficult and time-consuming, and at this phase of development we need to go as fast as possible. Keeping pools of untreated patients at hand for this purpose, and depriving them of currently available drugs, is ethically questionable.

It is easier and faster to work with healthy volunteers, and, better, young healthy volunteers. This requires the use of models, in which the putative drug is evaluated for its ability to reverse either induced cognitive impair-

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Selected abbreviations and acronyms

ACh	<i>acetylcholine</i>
AChR	<i>acetylcholine receptor</i>
AD	<i>Alzheimer's disease</i>
APP	<i>amyloid precursor protein</i>
βA	<i>β-amyloid</i>
CAT	<i>choline acetyltransferase</i>
NMDA	<i>N-methyl-D-aspartate</i>
NPY	<i>neuropeptide Y</i>

ment or associated markers (using electroencephalogram [EEG], positron emission tomography [PET] scan, and functional magnetic resonance imaging [fMRI] changes), or both.

The scopolamine model

Scopolamine is a nonselective,¹ competitive² muscarinic receptor blocker. The scopolamine model has its roots in the cholinergic hypothesis of aging and AD, and has played a major role in its construction, which we will recall briefly here.

From the beginning of the 20th century until the mid-fifties, scopolamine was used in obstetrics to induce a twilight state and amnesia during childbirth.³ In the sixties and seventies, it became obvious that regions rich in cholinergic afferents, such as the hippocampus, were involved in memory processes (see reference 4 for a review). In 1965, acetylcholine esterase activity was shown to be lowered in AD.⁵ In 1974, Drachman and Leavitt⁶ administered scopolamine to healthy young volunteers, who then displayed a memory profile very close to that observed in elderly people.

Two to three years later, three independent research teams⁷⁻⁹ reported a decreased activity of choline acetyltransferase (CAT), the enzyme responsible for acetylcholine (ACh) synthesis, in the cortex of AD patients. This decrease was shown to be correlated with brain lesions and clinical status.^{10,11} It was soon found that neuronal loss occurs in the forebrain basal nucleus of Meynert¹² and medial septal nucleus,¹³ which are the source of neocortical and hippocampal cholinergic afferent fibers, respectively.¹⁴⁻¹⁶ In its early version,⁴ the cholinergic hypothesis stated nothing about etiological factors, did

not address the additional roles that ACh dysfunction may play in other neurobehavioral disturbances of aging and dementia, and did not imply any exclusive or solitary involvement of the cholinergic system in age-related memory loss. It was a kind of “black box” model, in which an unknown pathophysiological process induces deficiency in various neurotransmission pathways thought to be responsible for the cognitive and behavioral aspects of aging and dementia. Despite obvious shortcomings (see references 17–20 for review and discussion), the cholinergic hypothesis legitimized the development of the cholinergic drugs we prescribe today and the administration of scopolamine as a model of investigation for AD research.

The scopolamine model was used in cognitive research to study the clinical correlates of ACh deficiency (see reference 21 for a review). It was applied to elderly subjects and AD patients²²⁻³³ as a marker of cholinergic sensitivity, with the purpose of improving the diagnosis and staging of the disease. It failed, however, to predict cognitive decline on the basis of the subjects' sensitivity.³⁴

Animal studies assessing the reversal of scopolamine-induced memory impairment by various compounds are too numerous to be cited exhaustively. This approach has also been used in humans with the following molecules: physostigmine,³⁵⁻⁴⁰ velnacrine,⁴⁰ choline,⁴¹ RO 15-1788,³⁹ moclobemide,^{42,43} RU 41656,⁴⁴ L- α -glycerylphosphorylcholine,⁴⁵ oxiracetam,⁴⁶ aniracetam and piracetam,⁴⁷ tenilsetam,⁴⁸ BMY 21502,⁴⁹ D-cycloserine,⁵⁰ SDZ ENS-163,⁵¹ and ZK-93426.⁵² However, the scopolamine model has not become a standard tool in the early assessment of drugs.

One reason for this is that the cognitive changes induced by scopolamine do not really mimic the AD picture. The details of the differences listed in *Figure 1* (based on references 28, 40, and 53–63) are open to discussion, but there is a general agreement on the fact that, as Wesnes⁴⁰ wrote, all the scopolamine-induced deficiencies are also observed in AD, while the reverse is not always true. The same is observed in neurological investigations. The electrophysiological effects of scopolamine (reviewed in reference 64) are close on EEG and similar on visual evoked potentials to those of AD. In PET⁶⁵⁻⁶⁸ and single photon emission computed tomography (SPECT)⁶⁹ studies, scopolamine induces cerebral blood flow (CBF) and glucose metabolism changes, which are sometimes divergent and region-specific, but in all cases different from the pattern observed in AD.

The ketamine model




Ketamine is a noncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonist.^{70,71} Its administration in order to produce a model is the correlate of the glutamatergic hypothesis of AD (reviewed in reference 72). Two, apparently opposite, glutamatergic hypotheses have been proposed. The excitotoxic hypothesis states that there is a glutamatergic hyperactivity in AD. Domoic acid poisoning in humans was responsible for irreversible memory loss.⁷³ Neuronal⁷⁴ and astroglial⁷⁵ glutamate transporter dysfunction in AD could result in excess glutamate in the synaptic cleft and in excitotoxic neuronal damage. This hypothesis is consistent with the beneficial effects of memantine⁷⁶ and lamotrigine⁷⁷ in AD patients. Some findings provide a link with the histopathological lesions that are the hallmarks of AD. Kainic acid injection in the rat leads to decreased neuronal amyloid precursor protein (APP) 695 mRNA owing to neuronal death, and increased astrocytic APP 770 mRNA,⁷⁸ which has been found selectively increased in AD in comparison with other neurodegenerative disorders⁷⁹ and associated with plaques.⁸⁰ β -Amyloid sensitizes neurons to glutamate toxicity⁸¹ and also enhances glutamate release by macrophages.⁸² Furthermore, in neuronal culture, glutamate was shown to enhance tau gene expression⁸³ and induce paired helical filaments similar to those found in AD.⁸⁴ The hypoglutamatergic hypothesis has been extensively reviewed elsewhere (see Newcomer et al, in this issue). The ketamine model is its application. Ketamine is mainly used in the field of schizophrenia research to provoke psychotomimetic as well as cognitive effects.⁸⁵⁻⁹⁴ These studies did not all assess the same functions or use the same paradigm to assess a particular function. Despite this limitation, when these studies are summarized and the profile of ketamine effects compared with that of AD (Figure 1),⁶⁰⁻⁶³ the situation is the same as that for the scopolamine model: the functions affected by ketamine are affected in AD, but the reverse is not necessarily the case.

Future directions

The two main models proposed thus lead to some of the attentional and memory impairment observed in AD, but do not fully reproduce the AD pattern. Two options are therefore possible. Since multiple neuro-

	AD	SCOPOLAMINE	KETAMINE
Immediate memory	↓	↔	Controversial
Working memory	↓	Controversial	↓
Encoding	↓	Controversial	↔
Storage	↓	↓	↓
Consolidation	↓	↓	↓
Explicit memory			
<i>Episodic</i>	↓	↓	↓
<i>Semantic</i>	↓	↓	Controversial
Implicit memory			
<i>Procedural</i>	↔	NA	NA
<i>Priming</i>	↔	↔	NA
Free recall	↓	↓	↓
Cued recall	↓	↔	NA
Recognition	↓	↓	↓
Delayed recall	↓	↓	↓
Learning capacity	↓	↓	Controversial

Figure 1. Memory dysfunction in Alzheimer's disease (AD) and after scopolamine or ketamine.

 slightly affected;
  not affected;
  severely affected.

NA: not assessed.

transmitter systems are affected in AD, it has been suggested⁹⁵ that combination modeling through simultaneous administration of drugs that impair several neurotransmitters or different aspects of a single system could mimic the AD pattern more closely. The few published studies on this strategy add mecamlamine,⁹⁶ *m*-chlorophenylpiperazine (mCPP),⁹⁷ metergoline, or haloperidol⁹⁸ to scopolamine, and report no⁹⁸ or marginal^{96,97} cumulative effect. Although NMDA antagonists were shown to potentiate the amnesic effect of scopolamine in the rat,⁵⁸ no study on this combination in humans has been published to date. Beyond the

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weakness of their effects, combined models are so complex that they become difficult to understand—and particularly difficult to manipulate—in the assessment of cognitive enhancers. Another method is to take advantage of the recent advances in our understanding of AD. Currently available data support the view that neuronal and synaptic loss, rather than secondary neurotransmission disruption, is most likely responsible for cognitive changes in AD.⁹⁹ They also allow attempts to integrate neurotransmitter changes into a more comprehensive theoretical framework. The cholinergic hypothesis in its current version (*Figure 2*) focuses on the reciprocal modulatory influences of cholinergic transmission and APP processing (reviewed in references 100 and 101). β -Amyloid (β A) is known to be neurotoxic at high (micromolar) concentrations.¹⁰² In vitro, soluble β A at picomolar to nanomolar levels is not toxic but does inhibit synthesis and stimulated release of ACh.¹⁰³⁻¹⁰⁵ Studies on the possible inhibitory effect of β A on CAT activity¹⁰⁴⁻¹⁰⁶ gave divergent results. β A appears to exert its effect on ACh synthesis and release through depletion of ACh precursors. It has been shown to disrupt the activity of pyruvate dehydrogenase,¹⁰⁶ which generates acetyl coenzyme A (CoA) from pyruvate and was found to be decreased in the cortex of AD patients,¹⁰⁷ and to inhibit high-affinity choline uptake.¹⁰⁴ This could have an indirect neurotoxic effect, since cholinergic neurons deprived of choline have been shown to break down phosphatidylcholine from intracellular organelle membranes to provide additional choline.¹⁰⁸ Although there is no general consensus (see reference 109 for review), it is thought that postsynaptic muscarinic M_1 acetylcholine receptor (AChR) density is unchanged in AD, while those of presynaptic M_2 and nicotinic AChRs are reduced.^{110,111} It has been shown that activation of protein kinase C through M_1 (and M_3) AChRs lowers β A production by favoring the nonamyloidogenic processing of APP.^{112,113} Despite their unchanged density, M_1 receptors could be dysfunctional^{114,115} because of defective coupling to Gq/11 proteins.¹¹⁶ This could lead to increased β A production, which would further impair M_1 AChR signal transduction.¹¹⁷ M_1 AChR-G protein uncoupling could also favor protein tau phosphorylation and thus paired helical filament formation through disinhibition of mitogen-activated protein (MAP) kinases¹¹⁸ and decreased efficiency of tau dephosphorylation.¹¹⁹

Despite the uncertainties that remain, it is clear that the cholinergic deficiency can no longer be seen as a late consequence of neuropathological changes, but at least as a contributor to the cascade of events leading to full-blown dementia.

The glutamatergic hypothesis has also been revisited. Its current version (see Newcomer et al, in this issue) reconciles the former hyper- and hypoglutamatergic hypotheses by proposing a two-stage process.

- In the first stage, β A increases the sensitivity of NMDA receptors to normal concentrations of glutamate, leading to destruction of NMDA-bearing GABAergic (GABA: gamma-aminobutyric acid), noradrenergic (NE), and serotonergic (5-HT) neurons, which have an inhibitory action on basal forebrain cholinergic, anterior thalamic glutamatergic, and cortical neuropeptide Y (NPY) neurons either directly (GABA, NE) or through activation of GABA neurons (5-HT).
- This loss of inhibition leads to hyperstimulation of cortical and corticolimbic neurons, which then degenerate, as do the hyperactive cholinergic, glutamatergic, and NPY neurons.

This suggests that for both cholinergic and glutamatergic neurons, the hypoactive, symptomatic stage is preceded by a hyperactive one. These new versions of the cholinergic and glutamatergic hypotheses make it necessary for us to reappraise our models. The goal of an acute pharmacological model is to transiently reproduce the hypoactive, symptomatic stage. According to the scheme proposed by Newcomer et al elsewhere in this issue, NMDA blockers induce transient hyperactivity of basal forebrain cholinergic, anterior thalamic glutamatergic, and cortical NPY neurons. It is likely that the mechanism by which acute administration of NMDA blockers produces memory impairment is different and does not involve the two-stage sequence proposed as a chronic model. The finding that pretreatment with haloperidol reduces ketamine-induced impairment in executive cognitive functions⁹¹ nonetheless suggests that the cognitive effect of NMDA blockade is indirect and nonselective. Higher selectivity, which would also avoid psychotomimetic symptomatology, might be achieved by acting downstream of the NMDA receptor. For the particular posterior cingulate and retrosplenial region, the best choice would be to give m_3 and/or kainate receptor blockers. Another target of choice is the hippocampus,

in which the most common muscarinic receptor is the m_1 subtype; the m_2 subtype represents 15% and the m_3 subtype globally less than 12%.¹²⁰ Moreover, specific blockade of the m_1 receptors would best reproduce their status in AD, where they are hypostimulated (because of presynaptic neuronal loss) and dysfunctional. The only molecule which is more or less selective for the m_1 receptor¹²¹ and available for human use is pirenzepine. It is said to cross the blood–brain barrier poorly,¹²² but very few studies have assessed its central effects in man^{123–125} and we think it deserves further study.

Do neurotransmitter-based pharmacological models have a future?

The way the cognitive symptoms are produced in AD is complex and many therapeutic strategies already in development address β A metabolism and toxicity,^{126–128} rather than cholinergic deficiency. However, D-cycloserine, which does not act on the cholinergic system but modulates the NMDA receptor, has been shown to attenuate the effect of scopolamine on memory.⁵⁰ Moclobemide, a selective monoamine oxidase A (MAOA) inhibitor,^{42,43} and thyrotropin-releasing hor-

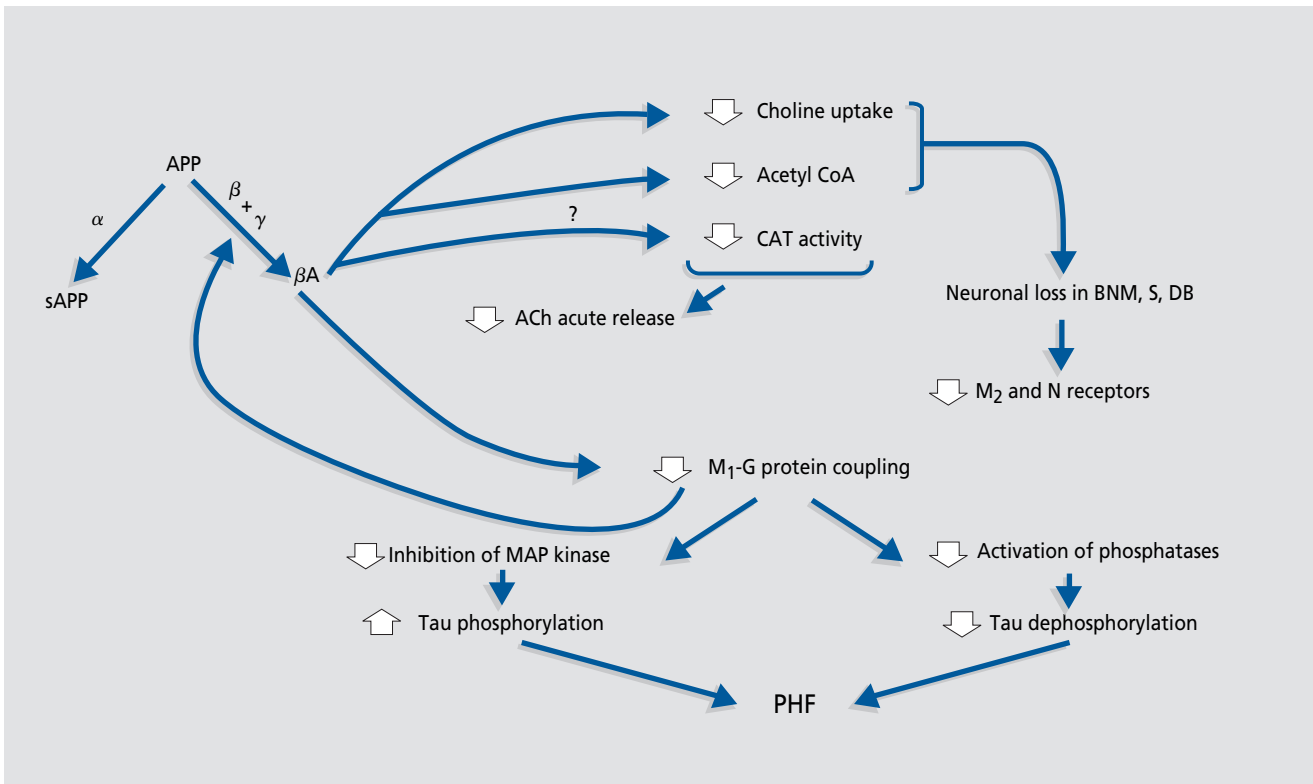


Figure 2. The cholinergic hypothesis today.

Amyloid precursor protein (APP) is processed either by α -secretase into a nonamyloidogenic pathway or by β - and γ -secretases to produce β -amyloid peptide (β A). β A could decrease choline acetyltransferase (CAT, the acetylcholine synthesis enzyme) activity. It lowers the availability of the substrates for acetylcholine (ACh) synthesis by impairing high-affinity choline uptake and acetyl coenzyme A (acetyl CoA) production; therefore ACh release is also diminished. Choline deprivation could initiate the so-called "autocannibalism" process through which ACh neurons break down membrane phosphatidylcholine to increase choline availability. Autocannibalism could be partly responsible for neuronal loss in the basal nucleus of Meynert (BNM), medial septal nucleus (S), and nucleus of the diagonal band of Broca (DB), and for the observed decrease in muscarinic M_2 and nicotinic (N) receptor densities, which are mainly presynaptic. Muscarinic M_1 receptors are mainly postsynaptic and their density is not affected in Alzheimer's disease. However, they are probably dysfunctional because of receptor–G protein uncoupling, with two consequences: (i) lowered M_1 signal transduction favors the amyloidogenic APP processing pathway, which further aggravates uncoupling; and (ii) through loss of inhibition of mitogen-activated protein (MAP) kinase, which results in increased tau protein phosphorylation, and inhibition of phosphatase, which results in a lesser dephosphorylation of tau, it favors the formation of paired helical filaments (PHF).

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mone (TRH)¹²⁹ were also able to partly reverse the scopolamine-induced deficits. In the animal, the same has been observed with estrogens¹³⁰ and G_{MI} gangliosides.¹³¹ Given these data and the current view that we have on the involvement of the cholinergic deficiency, it is very possible that new compounds, which do not act directly on the cholinergic system, could be effective on cholinergic models. Neurotransmitter-based models still have a place in our armamentarium, although efforts should be made to develop other approaches.

Conclusion

Whatever the model chosen, we must admit that it is impossible to reproduce the full AD cognitive pattern. Instead, we can try to produce some aspects of memory impairment, which is considered as the core symptom of the disease. The method used should be as simple and selective as possible in order to allow its manipulation. Selective ligands are currently in development¹³²; accelerating the toxicological studies of these compounds could allow us to work this way in the near future. □

Modelos farmacológicos en la investigación de la enfermedad de Alzheimer

Un empleo más generalizado de modelos farmacológicos facilitaría el desarrollo de nuevos fármacos para la enfermedad de Alzheimer (EA). Los dos modelos principales actualmente en uso se basan en las hipótesis colinérgica y glutamatergica de la EA. Aunque ambos modelos se orientan hacia algunos de los deterioros en la atención y en la memoria observados en la EA, ellos no reproducen totalmente el patrón de la EA. Los pocos estudios que utilizan una combinación de modelos (ej. la administración simultánea de varios fármacos con el objetivo de afectar algunos neurotransmisores o diferentes aspectos de un sistema único) no han informado de ningún efecto significativo, a lo más el efecto acumulativo es marginal. De acuerdo con el conocimiento actual del compromiso del glutamato y de la acetilcolina en la fisiopatología de la EA, nosotros sugerimos que los modelos que emplean bloqueadores selectivos de los receptores muscarínicos-1 (M₁) podrían imitar mejor la situación del sistema colinérgico en la EA. Esta clase de modelo sería conveniente para la evaluación de fármacos que no actúan directamente en el sistema colinérgico.

Modèles pharmacologiques dans la maladie d'Alzheimer

Le recours plus fréquent aux modèles pharmacologiques devrait faciliter le développement de nouvelles thérapeutiques dans la maladie d'Alzheimer (MA). Les deux principaux modèles utilisés actuellement sont fondés sur l'hypothèse d'une implication du système cholinergique et du système glutamatergique dans la maladie d'Alzheimer. Mais si l'on peut observer dans ces modèles l'apparition de certains des troubles de l'attention et de la mémoire observés au cours de la MA, ils ne génèrent pas la totalité des manifestations de la pathologie. Dans les quelques études qui ont utilisé un modèle combinant l'administration simultanée de plusieurs molécules dans le but d'altérer différents neurotransmetteurs ou différentes étapes d'un même système, l'effet cumulatif obtenu a été nul ou négligeable. En nous appuyant sur nos connaissances actuelles de l'implication du glutamate et de l'acétylcholine dans la physiopathologie de la MA, nous proposons d'utiliser des inhibiteurs sélectifs des récepteurs muscariniques-1 (M₁) pour reproduire au mieux l'état du système cholinergique dans la MA. Ce type de modèle pourrait permettre d'évaluer des molécules qui n'agissent pas directement sur le système cholinergique.

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